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ASSISTANT COMMISSIONER FOR PATENTS
BOX PATENT APPLICATION
Washington, D.C. 20231

Sir:

Transmitted herewith for filing under 37 CFR 1.53(b) is the
    [ ] patent application of
    [ ] continuation patent application of
    [ ] divisional patent application of
    [X] continuation-in-part patent application of U.S. Patent
Application 08/788,822, filed 1/23/97.

Inventor(s)/Applicant Identifier: Alessandro Sette

Federico Gaeta, Howard M. Grey, John Sidney, Jeffery L. Alexander

For: INDUCTION OF IMMUNE RESPONSE AGAINST DESIRED DETERMINANTS

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I hereby certify that this is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above, addressed to:

Assistant Commissioner for Patents
Washington, D.C. 20231

By: _Joy M. Marshall_

[X] This application claims priority from each of the following Application Nos./filing dates:
    This application is a continuation-in-part of U.S. Patent Application No. 08/788,822, filed January 23, 1997, which application claims benefit of U.S. Provisional Application No. 60/010,510, filed January 24, 1996, now abandoned. This application is also a continuation-in-part of U.S. Patent Application No. 09/310,462, filed May 12, 1999, now pending, which is a continuation-in-part of U.S. Patent Application No. 08/485,218, filed June 7, 1995, now abandoned, which is a continuation-in-part of U.S. Patent Application No. 08/305,871, filed September 14, 1994, now Patent No. 5,736,142, which is a continuation-in-part of U.S. Patent Application No. 08/121,101, filed Septebmer 14, 1993, now abandoned.   , the disclosure(s) of which is (are) incorporated by reference.

Enclosed are:
    [X]   52   page(s) of specification
    [X]   11   page(s) of claims
    [X]   1   page of Abstract
    [X]   A [ ] signed [X] unsigned Declaration.
    [X]   Preliminary Amendment

Applicant requests deferral of the filing fee until submission of the Missing Parts of Application.

Accordingly, please do not charge the filing fee at this time.

Please charge Deposit Account No. 20-1430 as follows:
    [ ]   Filing fee $_____
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Respectfully submitted,
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

    Alessandro Sette

Application No.: Not assigned

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For: INDUCTION OF IMMUNE
RESPONSE AGAINST DESIRED
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PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

    Prior to examination of the above-referenced application, please enter the following amendments and remarks.

IN THE CLAIMS:

    Please cancel claims 1-77.

    Please add claims 78-83 as follows:

    78.   A peptide of fewer than 30 amino acids, which binds more than one DR allele, the peptide comprising a sequence of the formula R_1-R_2-R_3-R_4-R_5, proceeding from the amino terminus to the carboxyl terminus, wherein

    R_1 is a D- or an L-amino acid followed by alanine or lysine;

    R_2 is selected from the group consisting of cyclohexylalanine, tyrosine and phenylalanine;

    R_3 is 3 or 4 amino acids, wherein each amino acid is independent selected from the group consisting of alanine, isoleucine, serine, glutamic acid and valine;

    R_4 is tryptophan-threonine-leucine-lysine; and

R_5 consists of 2 to 4 amino acids followed by a D- or an L-amino acid residue, wherein each of the 2 to 4 amino acids is independently selected from the group consisting of alanine, serine and valine.

79.   The peptide of claim 78, wherein the amino terminus and the carboxyl terminus of the peptide comprise D-amino acids.

80.   A peptide of fewer than 30 amino acids, which peptide binds more than one DR allele, the peptide comprising a sequence of the formula $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, wherein:

X_1 is a D- or L-amino acid;

X_2 is an amino acid selected from the group consisting of: A and K;

X_3 is an amino acid selected from the group consisting of: (X), Y or F, wherein (X) is cyclohexylalanine;

X_4, X_5 and X_6 are amino acids independently selected from the group consisting of: A, I, S and V;

X_7 is the amino acid W;

X_8 is the amino acid T;

X_9 is the amino acid L;

X_{10} is the amino acid K;

X_{11} and X_{12} are amino acids independently selected from the group consisting of: A, S and V; and

X_{13} is a D- or L-amino acid.

81.   The composition of claim 80, wherein the peptide further comprises X_{14}, which is a D- or L-amino acid; and

X_{13} is an amino acid selected from the group consisting of A, S and V and.

82.   The composition of claim 81, wherein the peptide further comprises X_{15}, which is a D- or L-amino acid; and

X_{14} is an amino acid selected from the group consisting of A, S and V.

83.   The peptide of claim 80, wherein the amino terminus and the carboxyl terminus of the peptide comprise D-amino acids.

REMARKS

With this Amendment, claims 1-77 are canceled without prejudice to later prosecution. Claims 78-83 are added and pending with entry of this Amendment.

The specification of the present application is identical to U.S. Patent Application No. 08/788,822, filed January 23, 1997, with two exceptions. First, the title of the present application is different than the title of U.S. Patent Application No. 08/788,822. Second, the present application claims priority, and incorporates by reference U.S. Patent Application No. 08/788,822.

Support for new claims 78-83 can be found in U.S. Patent Application No. 08/305,871, filed September 14, 1994, now U.S. Patent No. 5,736,142 ("the '142 patent"). The '142 patent is a parent application of, and is incorporated by reference by, the present application. All support is therefore provided with reference to the '142 patent.

For example, support for new claim 78 can be found on column 3, lines 1-19 of the '142 patent, which describes claimed pan DR peptides. In light of the fact that the pan DR peptides of the invention can be synthesized via recombinant DNA techniques (column 6, lines 54-65), it is clear that pan DR peptides need not include D-amino acids. *See, also*, column 10, lines 1-3 (stating that peptides of the invention "may generally comprise either L-amino acid or D-amino acids"). Indeed, claim 34 of the '142 patent is directed to pan DR peptides with the exact same amino acid sequences recited in claim 78 of the present application, with the exception that the sequences of claim 78 are limited to those embodiments where R_4 is tryptophan-threonine-leucine-lysine. Support for "a peptide of fewer than 30 amino acids can be found, e.g., on column 4, lines 5-6 of the '142 patent.

Support for claims 79 and 83 can be found, e.g., on column 9, lines 36-39, which states that L-amino acid-containing peptides can be capped with a D-amino acid.

Support for claim 80-82 can be found essentially the same place in the '142 patent as the support for claim 78. One difference between claims 80-82 and claim 78 is the nomenclature used to describe the claimed peptides. This nomenclature has been provided merely to facilitate examination and description of the claimed peptides and does not introduce new matter. The main advantage of this new nomenclature is that every amino acid is defined by its own variable (e.g., "X_1") whereas the variables used in claim 78 (e.g., R_1") often provide

for more than one amino acid residue. By providing one variable for each amino acid position of a peptide, it is significantly easier to determine the claimed subject matter. Thus, the new nomenclature does not represent new matter as it does not change the genus of peptides encompassed by the claim.

The peptides represented by claim 80 correspond to a subset of peptides claimed in claim 78. The following table provides a comparison between the peptides recited in claims 78 and 80.

Claim 80	Claim 78
X_1: A D- or L-amino acid	R_1: A D- or L-amino acid followed by A or K
X_2: A or K	
X_3: (X), Y or F, wherein (X) is cyclohexylalanine	R_2: (X), Y or F, wherein (X) is cyclohexylalanine
X_4: A, I, S or V	R_3: 3 or 4 amino acids selected from A, I, S, E or V
X_5: A, I, S or V	
X_6: A, I, S or V	
X_7: W	R_4: WTLK
X_8: T	
X_9: L	
X_{10}: K	
X_{11}: A, S or V	R_5: 2-4 amino acids selected from A, S or V followed by a D- or L-amino acid
X_{12}: A, S or V	
X_{13}: A D- or L-amino acid	

There are two substantive differences between the claims of claim 78 and claim 80. First, claim 78 provides that R_3 is 3 or 4 amino acids, whereas claim 80 corresponds to R_3 as 3 amino acids only. Second, claim 78 provides that R_5 is 2-4 amino acids whereas claim 80 provides two amino acids (i.e., X_{11} and X_{12}). Thus, in each aspect, claim 80 includes one of the species provided in the Markush groups of claim 78.

The additional limitations provided in dependent claims 81-82 correspond to the same subject matter in the "R_5" position of claim 78, wherein R_5 is 3 or 4 amino acids, respectively.

In light of the above explanation, Applicants submit that no new matter is introduced by this amendment.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

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United States Patent Application

INDUCTION OF IMMUNE RESPONSE AGAINST DESIRED DETERMINANTS

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INDUCTION OF IMMUNE RESPONSE AGAINST DESIRED DETERMINANTS

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STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

CROSS-REFERENCE TO RELATED APPLICATIONS

10   This application is a continuation-in-part of U.S. Patent Application No. 08/788,822, filed January 23, 1997, now pending, which application claims benefit of U.S. Provisional Application No. 60/010,510, filed January 24, 1996, now abandoned. This application is also a continuation-in-part of U.S. Patent Application No. 09/310,462, filed May 12, 1999, now pending, which application is a continuation-in-part of U.S. Patent

15   Application No. 08/485,218, filed June 7, 1995, now abandoned, which application is a continuation-in-part of U.S. Patent Application No. 08/305,871, filed September 14, 1994, now Patent No. 5,736,142, which application is a continuation-in-part of U.S. Patent Application No. 08/121,101, filed September 14, 1993, now abandoned. Each of these applications is incorporated herein by reference for all purposes.

20   BACKGROUND OF THE INVENTION

Field of the Invention

This invention pertains to the field of compounds and compositions useful for eliciting and/or enhancing an immune response.

Background

25   MHC molecules are classified as either class I or class II molecules. Class II MHC molecules are expressed by specialized antigen presenting cells (APC) such as

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macrophages, dendritic cells, or B cells. The class II MHC molecules usually associate with peptide fragments derived from processing of protein antigens which enter an endocytic pathway from the APC exterior. The MHC-peptide complexes are subsequently presented for scrutiny to $CD4^+$ T helper cells which can then become activated, proliferate and amplify

5   the immune response to the particular immunogenic peptide that is displayed. Accordingly, activation of T cells requires engagement of the T cell receptor (TCR) by its ligand, namely, a bi-molecular complex of an MHC molecule and a peptide antigen (Shimonkevitz, *et al.*, *J. Immunol.* 133, 2067-2074 (1984); Babbitt, *et al.*, *Nature* 317, 359-361 (1985); Buus, *et al.*, *Cell* 47, 1071-1077 (1986); Townsend, A., and Bodmer, H., *Annu. Rev. Immunol.* 7,

10   601-624).

Immunogenic peptides that contain epitopes recognized by T helper cells have been found to be useful in inducing immune responses. The use of helper peptides to enhance antibody responses against particular determinants is described, for instance, in Hervas-Stubbs *et al. Vaccine* 12:867-871 (1994). The interaction of MHC molecules with T

15   cells, in particular helper T cells, is also involved in determining the nature of the immune response raised against a particular vaccine antigen, which is important to the overall effectiveness of the vaccine.

MHC molecules are also involved in the inappropriate activation of T cells, which is a component of a number of immunopathologies, such as autoimmunity, allograft

20   rejection and allergic responses. Exemplary autoimmune diseases include rheumatoid arthritis, multiple sclerosis, and myasthenia gravis. Allergic responses involving T cell activation include allergies to various pollens, dust mites and the like. In addition, foreign infectious diseases may cause immunopathology (*e.g.*, Lyme disease, hepatitis, LCMV, post-streptococcal endocarditis, or glomerulonephritis). Food hypersensitivities, such as celiac

25   disease and Crohn's disease, as well as other allergic diseases, have been associated with particular MHC molecules or suspected of having an autoimmune component. The most commonly used approach to treating these conditions is to suppress the immune system, typically by using immunosuppressive drugs. Another approach has been proposed for cases in which the MHC molecule associated with the condition is known, involving selective

30   blockade of a given MHC molecule. However, where a number of MHC restrictions are involved, approaches other than selective blockade must be found.

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Immunochemical studies of the requirements for peptide binding to class II molecules have been carried out. The binding motifs of several murine and human class II MHC molecules have been defined, and motif analysis by sequencing of naturally processed peptides has also recently been described for various class II types (Rudensky *et al.*, *Nature*
5   353, 622-627 (1991); Chicz *et al.*, *Nature* 358, 764-768 (1992); Hunt *et al.*, *Science* 256, 1817-1820 (1992); Rudensky *et al.*, *Nature* 359, 429-431 (1992)). In the case of DR molecules in particular, it has been shown that a large hydrophobic anchor engaging a corresponding hydrophobic pocket of the MHC binding groove is the most crucial determinant of peptide-DR interactions. Several other anchors play definite, albeit less
10 prominent roles and help determine allelic specificity. Recently it has also been emphasized that the peptide backbone of the C-terminal half of the peptide molecule is engaged in direct hydrogen bonding with the walls of the MHC binding groove.

Although allele-specific polymorphic residues that line the peptide binding pockets of MHC molecules tend to endow each molecule with the capacity to bind a unique
15 set of peptides, there are many instances in which a given peptide has been shown to bind to more than one MHC molecule. This has been best documented in the case of the human DR isotype, in which it has been noted that several DR HLA molecules appear to recognize similar peptide motifs. Independently, several investigators reported cross-reactive binding and/or recognition of certain epitopes in the context of multiple DR types, leading to the
20 concept that certain peptides might bind more than one DR molecule (Busch et al., *Int. Immunol.* 2, 443-451 (1990); Panina-Bordignon *et al.*, *Eur. J. Immunol.* 19, 2237-2242 (1989); Sinigaglia *et al.*, *Nature* 336, 778-780 (1988); O'Sullivan *et al.*, *J. Immunol.* 147, 2663-2669 (1991) Roache *et al.*, *J. Immunol.* 144, 1849-1856 (1991); Hill *et al.*, *J. Immunol.* 147, 189-197 (1991)). However, the previously reported epitopes may have the capacity to
25 bind to several DR molecules, but they are by no means universal.

SUMMARY OF THE INVENTION

The present invention provides methods for a rational approach for selecting or constructing peptides, called "pan DR binding peptides," that bind to multiple HLA class II HLA-DR molecules; the present invention also provides such pan DR molecules
30 themselves. In preferred embodiments, such pan DR binding peptides serve as potent

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immunostimulators that can be readily employed in vaccines and other therapeutic agents. In alternative embodiments, the peptides can be used to yield immuno-inhibition. The rational molecular design disclosed here enables the creation or selection of therapeutic and immunostimulating molecules that can be administered across a broad population--for example, up to 50-80% or more of a large fraction of the global population. In contrast, molecules that bind to one HLA DR molecule may only bind to and be immunostimulatory for DR molecules in 10-20% of the same population. Thus, these pan DR binding peptides are highly advantageous for vaccines or therapeutic agents to be used in diverse populations.

The invention thus provides core pan DR binding peptides, and nucleic acids encoding them, as well as derivatives of these peptides that can stimulate an MHC-mediated T cell response. These pan DR binding peptides share a specified pattern of amino acid residues at designated core HLA binding positions. Stimulatory pan DR binding peptides of the invention have, at at least some of the non-HLA binding core positions, an amino acid that is relatively large, charged, polar, and/or aromatic. Accordingly, the presence of amino acids at positions that interact with a T cell receptor increases the ability of the peptide to stimulate a T cell response.

In alternative embodiments, the approaches presented here permit the design of peptides that can inhibit an MHC-mediated T cell response. Inhibitory peptides generally include, at the non-HLA binding core residues, relatively small, non-polar, non-charged, non-aromatic amino acids, e.g., alanine. The presence of such amino acids at residues that would otherwise interact with a T cell receptor diminishes the ability of the peptide-MHC complex to interact with the T cell receptors and thereby stimulate T cell activation.

The present invention also provides methods of using the pan DR binding peptides. For example, one can use the stimulatory pan DR peptides to enhance an immune response against an administered immunogen. For example, the pan DR binding peptides can be conjugated with a CTL-inducing peptide or other antigen and administered to induce a CTL response against, e.g., cells that bear a tumor-associated antigen or virally infected cells. In another embodiment, the pan DR peptides are conjugated with antibody-inducing peptides or admixed with an antibody-inducing peptide. Alternatively, one can use the inhibitory pan DR peptides to block an immune response by preventing activation of helper T cells. Due to their cross-reactive class II binding capacity, the inhibitory pan DR binding

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peptides can be used as therapeutics in the inhibition of T cell mediated events involved in allograft rejection, allergic responses, autoimmunity, and the like.

DETAILED DESCRIPTION

Definitions

5   The nomenclature used to describe peptide compounds follows the conventional practice wherein the amino group is presented to the left (the N-terminus) and the carboxyl group to the right (the C-terminus) of each amino acid residue. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital

10   single letter or a capital first letter of a three-letter symbol, and the D-form for those amino acids having D-forms is represented by a lower case single letter or a lower case three letter symbol. Glycine has no asymmetric carbon atom and is simply referred to as "Gly" or G.

The "major histocompatibility complex" or "MHC" contains more than 100 genes in humans. The genes encoding the α and β chains of "MHC class II" molecules are

15   linked within the complex. The particular combination of MHC alleles found on an individual chromosome is known as an "MHC haplotype." In humans, there exist three pairs of MHC class II α- and β-chain genes. These are referred to as HLA-DR, HLA-DP and HLA-DQ. However, in many haplotypes, the HLA-DR cluster contains an extra β-chain gene whose product can pair with the DRα chain. This means that the three sets of genes

20   actually give rise to four types of MHC class II molecules. All of the class II molecules are capable of presenting antigens to T cells.

An "oligopeptide" or "peptide" as used herein refers to a chain of at least four amino acid or amino acid mimetics. In a preferred embodiment, a peptide of the invention has at least six, more preferably at least nine, and usually fewer than about fifty residues. In a

25   particular embodiment, the peptide has fewer than about twenty-five, and preferably fewer than fifteen, e.g., nine to fourteen residues. The oligopeptides or peptides can be a variety of lengths, either in their neutral (uncharged) forms or in forms which are salts, and either free of modifications such as glycosylation, side chain oxidation, or phosphorylation or containing these modifications, subject to the condition that the modification not destroy the

30   biological activity of the polypeptides as herein described.

When referring to an amino acid residue in a peptide, oligopeptide or protein the terms "amino acid residue," "amino acid" and "residue" are used interchangeably and, as used herein, mean an amino acid or amino acid mimetic joined covalently to at least one other amino acid or amino acid mimetic through an amide bond or amide bond mimetic.

5   As used herein, the term "amino acid," when unqualified, generally refers to an "L-amino acid" or L-amino acid mimetic, although D-amino acids and L-amino acids may be referred to collectively by the term "amino acid."

Although the peptides will preferably be substantially free of other naturally occurring proteins and fragments thereof, in some embodiments the peptides can be

10   synthetically conjugated to native fragments or particles.

As used herein, the term "biological activity" means the ability to bind an appropriate MHC molecule and thereby elicit or modulate an immune response. For example, in the case of peptides useful for stimulating immune responses, biological activity comprises inducing a T helper response, which in turn helps induce an immune response

15   against a target antigen or antigen mimetic. In the case of peptides useful for stimulating antibody responses, the peptide will induce a T helper response, which in turn helps induce a humoral response against the target antigen.

A "pan DR binding peptide" or a "PADRE™" peptide (Epimmune, Inc., San Diego, CA) of the invention is a peptide capable of binding multiple different DR molecules.

20   The pan DR binding peptides can bind to at least 3, more preferably at least 4, 5, or 6 different DR molecules, more preferably 7 or more of the most common DR molecules, more preferably 9 of the most common DR molecules (e.g., DR1, 2w2b, 2w2a, 3, 4w4, 4w14, 4w15, 5, 6, 7, 8, 9, 52a, 52b, 52c, and 53), or alternatively, 50% or more of a panel of DR molecules representative of greater than or equal to 75% of the human population,

25   preferably greater than or equal to 80% of the human population. Preferably the pan DR binding peptides are capable of binding one or more DQ molecules, for example, DQ 3.1.

Throughout this disclosure, results are expressed in terms of IC_{50}'s. Given the conditions in which the assays are run (i.e., limiting MHC and labeled peptide concentrations), these values approximate K_D values. It should be noted that IC_{50} values can

30   change, often dramatically, if the assay conditions are varied, and depending on the particular reagents used (e.g., MHC preparation, etc.). For example, excessive

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concentrations of MHC will increase the apparent measured IC_{50} of a given ligand. An
alternative way of expressing the binding data, to avoid these uncertainties, is as a relative
value to a reference peptide. The reference peptide is included in every assay. As a particular
assay becomes more, or less, sensitive, the IC_{50}'s of the peptides tested may change

5   somewhat. However, the binding relative to the reference peptide will not change. For
example, in an assay run under conditions such that the IC_{50} of the reference peptide
increases 10-fold, all IC_{50} values will also shift approximately 10-fold. Therefore, to avoid
ambiguities, the assessment of whether a peptide is a good, intermediate, weak, or negative
binder should be based on its IC_{50}, relative to the IC_{50} of the standard peptide.

10   If the IC_{50} of the standard peptide measured in a particular assay is different
from that reported in Table 1, then it should be understood that the threshold values used to
determine good, intermediate, weak, and negative binders should be modified by a
corresponding factor.

Table 1

Allele	Assay standard	Sequence	SEQ ID NO:	Avg. IC_{50} (nM)
DR1	HA 307-319	PKYVKQNTLKLAT	1	5
DR2w2b	MBP 78-101	GRTQDENPVWHFFKNIVTPRTPPP	2	9.1
DR3	MT 65 kd 3-13	YKTIAFDEEARR	3	250
DR4w4	HA 307-319	PKYVKQNTLKLAT	1	45
DR4w14	717.01 combinatorial	YARFQSQTTLKQKT	4	50
DR5	Tet Tox 830-843	QYIKANSKFIGITE	5	20
DR7	Tet Tox 830-843	QYIKANSKFIGITE	5	25
DR52a	Tet Tox 1272-1284	NGQIGNDPNRDIL	6	470
DRw53	717.01 combinatorial	YARFQSQTTLKQKT	4	58
Dr2w2a	Tet Tox 830-843	QYIKANSKFIGITE	5	20
DQ3.1	ROIV	YAHAAHAAHAAHAAHAA	7	15
IAb	ROIV	YAHAAHAAHAAHAAHAA	7	28
IAd	Ova 323-326	ISQAVHAAHAEINE	8	110
IEd	lambda rep 12-26	YLEDARRLKAIYEKKK	9	170
IAs	ROIV	YAHAAHAAHAAHAAHAA	7	54
IAk	HEL 46-61	YNTDGSTDYGILQINSR	10	20
IEk	lambda rep 12-26	YLEDARRLKAIYEKKK	9	28

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The PADRE peptides of the invention, in addition to promoting an immune
response against a second determinant, can in certain embodiments serve as target antigens
themselves. Thus, for instance, in the case in which the PADRE peptide is linked to a

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carbohydrate epitope, the immune response may be to both the PADRE peptide and the carbohydrate epitope.

As used herein, the term "immunogenic determinant" is any structure that can elicit, facilitate, or produce an immune response, for example carbohydrate epitopes, lipids, proteins, peptides, or combinations thereof. An "antigenic determinant" is a structure that is recognized by one or more products of the immune response (e.g., antibodies, T cell receptors, and the like). The same molecule can be both an antigenic determinant and an immunogenic determinant.

A "CTL epitope" of the present invention is one derived from selected epitopic regions of potential target antigens, such as tumor associated antigens, including, but not limited to, renal cell carcinoma, breast cancer, carcinoembryonic antigens, melanoma antigens such as MAGE, and prostate cancer related antigens; as well as infectious disease/pathogenic antigens such as hepatitis C antigens, Epstein-Barr virus antigens, HIV-1 and HIV-2 antigens, and papilloma virus antigens.

A "humoral response" of the present invention is an antibody-mediated immune response directed towards various regions of an antigenic determinant. One of skill will recognize that a humoral response can also be induced against a PADRE peptide, wherein the PADRE peptide would also be included with the determinant; in this embodiment, the elicited immune response can be against both the antibody inducing determinant and the PADRE peptide.

A "carbohydrate epitope" as used herein refers to a carbohydrate structure, present as a glycoconjugate, e.g., glycoprotein, glycopeptide, glycolipid, and the like, or a polysaccharide, oligosaccharide, or monosaccharide against which an immune response is desired. The carbohydrate epitope may induce a wide range of immune responses. One of skill will recognize that various carbohydrate structures exemplified herein can be variously modified according to standard methods, without adversely affecting antigenicity. For instance, the monosaccharide units of the saccharide may be variously substituted or even replaced with small organic molecules, which serve as mimetics for the monosaccharide.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. Thus, the peptides of the present invention, when isolated, do not contain

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materials normally associated with their *in situ* environment, *e.g.*, MHC molecules on antigen presenting cells. Even where a protein has been isolated to a homogeneous or dominant band, there are trace contaminants in the range of 5-10% of native protein which co-purify with the desired protein. Isolated peptides of this invention do not contain such endogenous co-purified protein.

5

A "linker" as used herein is any used to provide covalent linkage and spacing between two functional groups (*e.g.*, a PADRE peptide and a desired determinant). Typically, the linker comprises neutral molecules, such as aliphatic carbon chains, amino acids or amino acid mimetics, which are substantially uncharged under physiological conditions and may have linear or branched side chains. Linkers can also comprise surface-active molecules such as lipids or surfactants. In some cases, the linker may, itself, be immunogenic, although non-therapeutically directed. Various linkers useful in the invention are described in more detail, below. Additionally, the verbs "link" and "conjugate" are used interchangeably herein and refer to covalent attachment of two or more species.

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The term "T cell clone" refers to a group of T cells that are progeny of a single virgin lymphocyte and express identical T cell receptor proteins. The term "virgin" lymphocyte is used here as it is used in Stites *et al.*, *Basic and Clinical Immunology*, 8th Edition, Prentice Hall, Englewood Cliffs, New Jersey (1994).

A "T helper peptide" as used herein refers to a peptide recognized by the T cell receptor of T helper cells, generally when the peptide is in combination with an MHC class II molecule. The PADRE peptides of the present invention are T helper peptides.

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Description of the Preferred Embodiments

The present invention provides an approach for rational design of pan DR binding peptides, which peptides can bind multiple MHC DR molecules. In some embodiments, the invention provides peptides that inhibit DR-restricted T cell proliferation, while in other presently preferred embodiments the peptides of the invention act as T helper epitopes that provide help for humoral and/or cell-mediated immune responses. Methods for rational design of such pan DR binding peptides are included.

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The pan DR peptides of the invention provide significant advantages over previously available peptides for modulating MHC class II-mediated immune responses. By

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virtue of their ability to bind to many different DR molecules, the peptides of the invention are useful in modulating T cell responses in a large fraction of the human population. The peptides of the invention can therefore yield enhanced immunogenic or repressive activity, compared to naturally occurring peptides.

5   Also provided by the invention are methods of using the pan DR peptides to block an immune response by preventing activation of helper T cells. Because of their cross-reactive class II binding capacity, the pan DR binding peptides are used as therapeutics in the inhibition of T cell mediated events involved in allograft rejection, allergic responses, or autoimmunity. Alternatively, the pan DR peptides are useful as an adjuvant-like component

10   in any vaccine formulation. In this embodiment the PADRE molecules are used to enhance an immune response against an administered immunogen. For instance, the pan DR binding peptides are administered with CTL-inducing peptides to induce a CTL response against, *e.g.*, virally infected cells or cells expressing tumor associated antigens. Alternatively, the pan DR binding peptides are conjugated with a CTL-inducing peptide or carbohydrate and

15   administered to induce a CTL response. In another embodiment, the pan DR peptides are conjugated with antibody-inducing peptides or carbohydrates. In addition, the pan DR binding peptides can be admixed with an antibody-inducing peptide or carbohydrate. The use of helper peptides to enhance antibody responses against particular determinants is described, for instance, in Hervas-Stubbs *et al.*, *Vaccine* 12:867-871 (1994).

20   The peptides of the invention can be prepared in a wide variety of ways. Because of their relatively short size, the peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. *See, for example*, Stewart and Young, *Solid Phase Peptide Synthesis*, 2nd Ed., Pierce Chemical Co.

25   (Rockford, Ill., 1984).

          Alternatively, recombinant DNA technology can be employed wherein a nucleotide sequence which encodes an immunogenic peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression. These procedures are generally known in the art, as

30   described generally in Berger and Kimmel, *Guide to Molecular Cloning Techniques, Methods in Enzymology* 152 Academic Press, Inc., San Diego, CA (Berger); Sambrook *et al.*

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(1989) *Molecular Cloning: A Laboratory Manual* (2nd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY, (Sambrook *et al.*); *Current Protocols in Molecular Biology*, F.M. Ausubel *et al.*, eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1994 Supplement)

5 (Ausubel); Cashion *et al.*, U.S. patent number 5,017,478 (issued May 21, 1991); and Carr, European Patent No. 0,246,864. Thus, fusion proteins that comprise one or more peptide sequences of the invention can be used to present the appropriate T cell epitope.

As the coding sequence for peptides of the length contemplated herein can be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci

10 *et al.*, *J. Am. Chem. Soc.* 103: 3185 (1981), modification can be made simply by substituting the appropriate base(s) for those encoding the native peptide sequence. The coding sequence can then be provided with appropriate linkers and ligated into expression vectors commonly available in the art, and the vectors used to transform suitable hosts to produce the desired fusion protein. A number of such vectors and suitable host systems are now available. For

15 expression of the fusion proteins, the coding sequence will be provided with operably linked start and stop codons, promoter and terminator regions and usually a replication system to provide an expression vector for expression in the desired cellular host. For example, promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired coding sequence. The resulting

20 expression vectors are transformed into suitable bacterial hosts. Of course, yeast or mammalian cell hosts can also be used, employing suitable vectors and control sequences. It is, of course, appreciated by those of skill in the art that only those embodiments of the invention comprising naturally occurring L-amino acids can be encoded by nucleic acids.

A. *Rational Design of pan DR Peptides*

25 The invention provides pan DR peptides, and methods for the rational design of the pan DR peptides. The methods can be used to select a native sequence or design a pan DR peptide *de novo*, or to identify modifications that one can introduce into a starting peptide, *e.g.*, one that already binds to one or more DR molecules, in order to broaden the specificity of the starting peptide. Once a pan DR binding core is designed into the peptide,

30 the invention provides methods for rationally designing the remainder of the peptide so that,

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depending upon the particular modifications introduced, the peptide either inhibits or stimulates T cell-mediated immune responses.

1. Pan DR Binding Core

The pan DR binding ability of a peptide of the invention is determined by the presence of particular types of amino acids at positions in the peptide that are designated "critical contact sites" (i.e., those residues (or their functional equivalents) that must be present in the peptide so as to confer upon the peptide the ability to bind to one, and in accordance with the invention, preferably several MHC DR molecules). The binding of a peptide to an MHC DR molecule involves the critical interaction between the side chains of these peptide residues with pockets located in the peptide-binding groove of the MHC molecule.

The peptides of the invention that include as the critical contact residues the amino acids as set forth below bind to multiple HLA-DR molecules with at least intermediate affinity, for example. In presently preferred embodiments, the pan DR binding peptides can bind to at least 3, more preferably at least 4, 5, or 6 different DR molecules, more preferably 7 or more of the most common DR molecules, more preferably 9 of the most common DR molecules (e.g., DR1, 2w2b, 2w2a, 3, 4w4, 4w14, 4w15, 5, 6, 7, 8, 9, 52a, 52b, 52c, and 53), or alternatively, 50% or more of a panel of DR molecules representative of greater than or equal to 75% of the human population, preferably greater than or equal to 80% of the human population. The pan DR peptides also preferably bind one or more DQ molecules. Preferably, the peptides bind with high affinity (IC_{50} of less than about 500 nM). Methods for determining the ability of a peptide to bind to MHC molecules are known to those of skill in the art, and are described in, for example, International Application WO 92/02543.

Using the generic formula Z_n-$X_1X_2X_3X_4X_5X_6X_7X_8X_9$-$Z_c$ to represent a peptide (see, e.g., Table 4), the "critical contact sites" for pan DR binding are, according to the present invention, at positions X_1, X_2, and X_6. For pan DR binding, X_1 is an amino acid selected from the group consisting of cyclohexylalanine (X), Y, F, M, L, I, V and W, X_2 is an amino acid selected from the group consisting of I and V; and X_6 is an amino acid selected from the group consisting of T, V, M, S, A, C, P, L, and I. "Cyclohexylalanine" includes, but is not limited to, bicyclohexylalanine, dicyclohexylalanine and

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homocyclohexylalanine. Other molecules that can be used in place of cyclohexylalanine to achieve pan DR binding activity include, for example, 3-cyanophenylalanine, 4-cyanophenylalanine, 3,4-difluorophenylalanine, 3,5-difluorophenylalanine, diphenylalanine, 2-fluorophenylalanine, 4-fluorophenylalanine, homophenylalanine, 4-iodophenylalanine, 4-

5 methylphenylalanine, 2-napthylalanine, 4-nitrophenylalanine, 2-pyridylalanine, 3-pyridylalanine, 1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid, 4-thiazoylalanine, 3-trifluoromethylphenylalanine, and 4-trifluoromethylphenylalanine; these molecules are also represented by (X) as an amino acid symbol in the tables and peptides herein.

In a presently preferred embodiment, X_1 is cyclohexylalanine, Y, or F, and X_6

10 is T. In the above formula, X_3, X_4, X_5, X_7, X_8, and X_9 are each an amino acid, and Z_n and Z_c each independently comprise 1 to about 20 amino acids (*see*, Table 3). In some embodiments, Z_n and Z_c each comprise about 20 amino acids or less, 15 amino acids or less, about 10 amino acids or less, or about 5 amino acids or less. Z_n and Z_c, in some embodiments, are at least 2 amino acids in length.

15 In some embodiments (*see, e.g.,* Table 4), the pan DR peptides of the invention are described using the formula Z_n-$X_0X_1X_2X_3X_4X_5X_6X_7X_8X_9$-$Z_c$, wherein: X_0 is an amino acid selected from the group consisting of K and A; X_1 is an amino acid selected from the group consisting of (X), Y and F; X_2 is an amino acid selected from the group consisting of I and V; and X_6 is T. X_3, X_4, X_5, X_7, X_8, and X_9 are each an amino acid, and Z_n

20 and Z_c each comprise 1 to 20 amino acids (*see,* Table 4). In some embodiments, Z_n and Z_c each comprise about 20 amino acids or less, 15 amino acids or less, about 10 amino acids or less, or about 5 amino acids or less. Z_n and Z_c, in some embodiments, are at least 2 amino acids in length.

The identity of amino acids X_0, X_3, X_4, X_5, X_7, X_8, and X_9, and also Z_n and Z_c

25 positions can determine whether a peptide will inhibit or stimulate T cell mediated immune responses, as described below. Where the amino acid present at a particular position is not correlated to a particular biological activity desired for the peptide, these positions can be any amino acid. In any case, however, it is desirable to use at each position an amino acid that does not interfere with the pan DR binding ability of the peptide.

30 In some embodiments, the peptides of the invention have, at their amino- and/or carboxy- termini, one or more amino acids, or mimetics thereof, that are chosen such

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as to alter physical or chemical properties of the peptide. These residues, designated Z_n and Z_c in the formulas set forth herein, can be chosen so as to affect properties such as binding, stability, bioavailability, ease of linking, and the like. Z_n and Z_c are each typically between about zero and about 20 amino acids (or mimetics) in length, more preferably between about one and about 15 amino acids, or zero to about 10 amino acids in length. Generally, the total length of the pan DR peptides of the invention is between about nine and about 50 amino acids. More preferably, the pan DR peptides are between about nine and about 40 amino acids, and still more preferably are between about 12 and about 25 amino acids in length.

To identify degenerate DR-binding epitopes, peptides can be screened for binding to sequential panels of DR molecules. For example, peptides that are capable of binding multiple DR molecules can be identified by the use of combined DR1-4-7 motifs. The composition of suitable panels, and the phenotypic frequency of associated antigens, are shown in Table 2. (*See,* Imanishi, T. *et al.*, Allele and haplotype sequences for HLA and complement loci in various ethnic groups. In *Proceedings of the Eleventh International Histocompatibility Workshop and Conference*, Vol. 1, K. Tsuji, M. Aizaqa, and T. Sasazuki, *et al.* (Tokyo, Japan, Oxford University Press).) For example, all peptides can be initially tested for binding to the molecules in the primary panel (*e.g.,* DR1, DR4w4 and DR7 (*see, e.g.,* Southwood *et al.* (1998) *J. Immunol.* 160: 3363-3373). Next, whether the peptides that exhibit degenerate binding behavior for the primary panel would also bind other common DR types as well is examined. Only peptides that bind (for screening, a peptide is considered a "binder" if it has an IC_{50} of less than or equal to 1000 nM) to at least two of these three molecules are then tested for binding in the secondary assays (*e.g.,* DR2w2β1, DR2w2β2, DR6w19 and DR9). Finally, only peptides that bind to at least two of the four secondary panel molecules, and thus four of seven molecules in total, are screened for binding in the tertiary assays (*e.g.,* DR4w15, DR5w11 and DR8w2). If desired, peptides that bind to members of the tertiary panel can be tested for ability to bind to a quaternary panel (*e.g.,* DR3, DR6, DR8, DR9, and DR12).

Modifications of peptides with various amino acid mimetics or D-amino acids, for instance at the N- or C- termini, are useful for instance, in increasing the stability of the peptide *in vivo*. Such peptides can be synthesized as "inverso" or "retro-inverso" forms, that is, by replacing L-amino acids of a sequence with D-amino acids, or by reversing

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the sequence of the amino acids and replacing the L-amino acids with D-amino acids. As the D-peptides are substantially more resistant to peptidases, and therefore are more stable in serum and tissues compared to their L-peptide counterparts, the stability of D-peptides under physiological conditions may more than compensate for a difference in affinity compared to

5 the corresponding L-peptide. Further, L-amino acid-containing peptides with or without substitutions can be capped with a D-amino acid to inhibit exopeptidase destruction of the antigenic peptide. Accordingly, stability can be increased by introducing D-amino acid residues at the C and N termini of the peptide. Previous studies have indicated that the half-life of L-amino acid-containing peptides *in vivo* and *in vitro*, when incubated in

10 serum-containing medium, can be extended considerably by rendering the peptides resistant to exopeptidase activity by introducing D-amino acids at the C and N termini. Other methods, *e.g.*, amidation of the carboxy termini of the peptides, can also impart increased stability.

Stability can be assayed in a number of ways. For instance, peptidases and

15 various biological media, such as human plasma and serum, have been used to test stability. *See, e.g.*, Verhoef *et al.*, *Eur. J. Drug Metab. Pharmacokin.* 11, 291-302 (1986); Walter *et al.*, *Proc. Soc. Exp. Biol. Med.* 148, 98-103 (1975); Witter *et al.*, *Neuroendocrinology* 30, 377-381 (1980); Verhoef *et al.*, *J. Endocrinology* 110, 557-562 (1986); Handa *et al.*, *Eur. J. Pharmacol.* 70, 531-540 (1981); Bizzozero *et al.*, *Eur. J. Biochem.* 122, 251-258 (1982);

20 Chang, *Eur. J. Biochem.* 151, 217-224 (1985).

Table 2: HLA-DR Screening Panels

Screening Panel	Antigen	Alleles	Representative Assay		Phenotypic Frequencies					
			Allele	Alias	Cauc.	Blk.	Jpn.	Chn.	Hisp.	Avg.
Primary	DR1	DRB1*0101-03	DRB1*0101	(DR1)	18.5	8.4	10.7	4.5	10.1	10.4
	DR4	DRB1*0401-12	DRB1*0401	(DR4w4)	23.6	6.1	40.4	21.9	29.8	24.4
	DR7	DRB1*0701-02	DRB1*0701-02	(DR7)	22.6	11.1	1.0	15.0	16.6	14.0
	Panel Total				59.6	24.5	49.3	38.7	51.1	44.6
Secondary	DR2	DRB1*1501-03	DRB1*1501	(DR2w2β1)	19.9	14.8	30.9	22.0	15.0	20.5
	DR2	DRB5*0101	DRB5*0101	(DR2w2β2)	-	-	-	-	-	-
	DR9	DRB1*09011,09012	DRB1*0901	(DR9)	3.6	4.7	24.5	19.9	6.7	11.9
	DR13	DRB1*1301-06	DRB1*1302	(DR6w19)	21.7	16.5	14.6	12.2	10.5	15.1
	Panel Total				42.0	33.9	61.0	48.9	30.5	43.2
Tertiary	DR4	DRB1*0405	DRB1*0405	(DR4w15)	-	-	-	-	-	-
	DR8	DRB1*0801-5	DRB1*0802	(DR8w2)	5.5	10.9	25.0	10.7	23.3	15.1
	DR11	DRB1*1101-05	DRB1*1101	(DR5w11)	17.0	18.0	4.9	19.4	18.1	15.5
	Panel Total				22.0	27.8	29.2	29.0	39.0	29.4
Quaternary	DR3	DRB1*0301-2	DRB1*0301	(DR3w17)	17.7	19.5	0.4	7.3	14.4	11.9
	DR12	DRB1*1201-02	DRB1*1201	(DR5w12)	2.8	5.5	13.1	17.6	5.7	8.9
	Panel Total				20.2	24.4	13.5	24.2	19.7	20.4

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The peptides or analogs of the invention can be modified by altering the order or composition of certain residues, it being readily appreciated that certain amino acid residues essential for biological activity, *e.g.*, those at critical contact sites, may generally not be altered without an adverse effect on biological activity. The non-critical amino acids

5   need not be limited to those naturally occurring in proteins, such as L-α-amino acids, or their D-isomers, but may include non-protein (including artificial) amino acids as well, such as β-γ-δ-amino acids, as well as many derivatives of L-α-amino acids. Accordingly, a peptide of the present invention can generally comprise either L-amino acids or D-amino acids, but not D-amino acids or non-protein (*e.g.*, artificial) amino acids within a core binding region. If a

10   peptide has no D-amino acids or other stabilizing modifications, it is sometimes desirable to increase the length of the peptide to provide for increased stability. Thus, it is generally preferred that if only L-amino acids are included in a peptide, or are encoded by a nucleic acid, that the peptide have several additional amino acids in the Z_n and Z_c regions to adjust for potential degradation.

15   ## 2. *T Cell Inhibitory Peptides*

The invention also provides peptides, and methods for the rational design of such peptides, that can inhibit an antigen-specific T cell-mediated immune response *in vivo*. These peptides are based on a pan DR binding core as described above. Once one has designed a pan DR peptide by introducing the specified amino acids at the "critical contact

20   residues" for MHC binding, the peptide can be further designed so as to inhibit an immune response. The rational design of such inhibitory peptides involves the use of a non-polar, non-charged, non-aromatic, non-bulky amino acid at peptide positions other than those that are "critical contact residues." These residues are positioned at sites in the molecules that are capable of being involved in the interaction between the peptide when complexed to MHC

25   and a T cell receptor. The interactions between a peptide and a receptor generally involve the side chains of amino acids having a charge, hydrophobicity, or some other feature for which the receptor has a corresponding moiety that is able to interact with the side chain (*e.g.*, a charge opposite that of the side chain on the peptide). Accordingly, by using an amino acid that is identified or engineered so as to possess a relatively small, inconspicuous side

30   chain(s), the ability of the peptide to interact with the T cell receptor is reduced or eliminated.

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Again using the generic formula Z_n-$X_1X_2X_3X_4X_5X_6X_7X_8X_9$-$Z_c$ to represent a peptide, a T cell inhibitory peptide will have a binding core of critical contact residues as set forth above, and one or more of the residues that are not "critical contact residues", *i.e.*, a non-HLA binding pocket residues X_3, X_4, X_5, X_7, X_8, and X_9 will be a non-polar, non-charged, non-aromatic, non-bulky amino acid. Most preferably, all six of these residues are a non-polar, non-charged, non-aromatic, non-bulky amino acid (Table 3). Generally, at least one of these residues, more preferably at least two, still more preferably at least three, four, or five residues is a non-polar, non-charged, non-aromatic, non-bulky amino acid. X_5 in particular, either alone or in combination with other residues, is a non-polar, non-charged, non-aromatic, non-bulky amino acid (*e.g.*, A) in some preferred embodiments.

The non-polar, non-charged, non-aromatic, non-bulky amino acids used at the specified positions include, for example, alanine and other amino acids with these characteristics. Properties of amino acids, *e.g.*, charge, polarity, etc., are described in, for example, Creighton, ed. *Proteins: structures and molecular properties*, 2nd Ed. (Freeman & Co., New York, 1993); Page, David S., *Principles of biological chemistry*, 2nd Ed., (Willard Grant Press, Boston, 1981); and Stryer, Lubert, *Biochemistry*, 3rd Ed. (Freeman & Co., San Francisco, 1988). In a presently preferred embodiment, an alanine is used at these "non-critical" positions, which are associated with T cell receptor, rather than HLA pocket binding properties.

An increase in inhibitory activity can be achieved by including one or more such amino acids in Z_n and/or Z_c, particularly the amino acid residue that is immediately adjacent to the binding core. For example, in a preferred embodiment at least one of, and preferably both, of Z_n and Z_c is an alanine or other non-polar, non-charged, non-aromatic, non-bulky amino acid.

In some embodiments, the pan DR peptides of the invention are described using the formula Z_n-$X_0X_1X_2X_3X_4X_5X_6X_7X_8X_9$-$Z_c$. The peptides include, for pan DR binding ability, X_0, which is an amino acid selected from the group consisting of K and A; X_1, which is an amino acid selected from the group consisting of (X), Y and F; X_2, which is an amino acid selected from the group consisting of I and V; and X_6, which is T (*see*, Table 4). To obtain or design an inhibitory peptide, at least one and more preferably more of residues X_3, X_4, X_5, X_7, X_8, and X_9 are non-polar, non-charged, non-aromatic, non-bulky

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amino acids. In some preferred embodiments, at least X_5 is alanine, glycine, proline, or similar residues; alanine is a particularly preferred residue. For inhibitory activity, X_0 preferably is also a non-polar, non-charged, non-aromatic, non-bulky amino acid, in particular alanine.

5   Once an inhibitory peptide is designed using the methods of the invention, it is desirable to test the peptide to ensure that it exhibits pan DR binding ability and, if desired, DQ binding ability. Those peptides that do exhibit such binding ability are then tested for biological activity. The biological activity of the peptides obtained using the rational design methods described above can be assayed in a variety of systems. Typically,

10   the ability to inhibit antigen-specific T cell activation is tested. In one exemplary protocol, an excess of peptide is incubated with an antigen-presenting cell of known MHC expression, (*e.g.*, DR1) and a T cell clone of known antigen specificity (*e.g.*, tetanus toxin 830-843) and MHC restriction (again, DR1), and the antigenic peptide itself (*i.e.*, tetanus toxin 830-843). The assay culture is incubated for a sufficient time for T cell proliferation, such as four days,

15   and proliferation is then measured using standard procedures, such as pulsing with tritiated thymidine during the last 18 hours of incubation. The percent inhibition, compared to the controls which received no inhibitory peptide, is then calculated.

          The capacity of peptides to inhibit antigen presentation in an *in vitro* assay has been correlated to the capacity of the peptide to inhibit an immune response *in vivo*. *In*

20   *vivo* activity can be determined in animal models, for example, by administering an antigen known to be restricted to the particular MHC molecule recognized by the peptide, along with the new immunomodulatory peptide. T lymphocytes are subsequently removed from the animal and cultured with a dose range of antigen. Inhibition by the engineered peptide of stimulation by the control peptide is measured by conventional means, *e.g.*, pulsing with

25   [^{3}H]-thymidine, and comparing to appropriate controls. Certain experimental details will of course be apparent to the skilled artisan. *See also*, Adorini *et al.*, *Nature* 334, 623-625 (1988).

          A large number of cells with defined MHC molecules, particularly MHC class II molecules, are known and readily available from, for instance, the American Type

30   Culture Collection (*see, e.g.*, online catalog at www.atcc.org; "Catalogue of Cell Lines and Hybridomas," 8th edition (1994) Manassas, VA).

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Examples of pan DR binding peptides of the invention that can inhibit a T cell mediated immune response include, for example:

aA(X)VAAATLKAAa

aA(X)IAAATLKAAa.

3. T Cell Stimulatory Peptides

In other particularly preferred embodiments, the invention provides peptides, and methods for rational design of such peptides, that can act as helper epitopes for the induction of humoral and/or T cell mediated immune responses. The principle which underlies the design of T cell stimulatory peptides is the converse of that for T cell inhibitory peptides. Whereas the design of inhibitory peptides involves the replacement of amino acid side chains that have significant binding energy with a T cell receptor with amino acids that do not, a T cell stimulatory peptide will have at these T cell receptor binding positions more bulky, hydrophobic, or charged amino acids. The presence of such residues can increase the affinity of interaction between the peptides and T cell receptors, thus increasing the immunogenicity of the peptides when inducing T cell mediated responses.

The T cell stimulatory peptides of the invention have, again using the generic formula Z_n-$X_1X_2X_3X_4X_5X_6X_7X_8X_9$-$Z_c$, a "binding core" as set forth above (see, e.g., Table 4). The "critical contact sites" for pan DR binding are, according to the present invention, at positions X_1, X_2, and X_6. For pan DR binding, X_1 is an amino acid selected from the group consisting of cyclohexylalanine (X), Y, F, M, L, I, V and W, X_2 is an amino acid selected from the group consisting of I and V; and X_6 is an amino acid selected from the group consisting of T, V, M, S, A, C, P, L, and I. In the T cell stimulatory peptides, one or more of the non-HLA pocket residues X_3, X_4, X_5, X_7, X_8, and X_9 will be an amino acid that has a side chain having significant binding energy for a T cell receptor. For example, one or more of these amino acids will be polar, charged, aromatic, and/or bulky. Preferably, at least one, more preferably at least two, still more preferably at least three, four, or five, and most preferably all six of these residues are a polar, charged, aromatic, and/or bulky amino acid. In most preferred embodiments, each of X_3, X_4, X_5, X_7, X_8, and X_9 is a polar, charged, aromatic, and/or bulky amino acid. In preferred embodiments, at least X_5 is such an amino acid, either alone or in combination with other amino acids. Moreover, an increase in stimulatory activity can be achieved by including one or more such amino acids in Z_n and/or

Z_c, particularly the amino acid residue that is immediately adjacent to the binding core. *See* Table 3 for a listing of amino acids that are preferred at the particular positions.

In some embodiments, the pan DR stimulatory peptides of the invention are described using the formula Z_n-$X_0X_1X_2X_3X_4X_5X_6X_7X_8X_9$-$Z_c$. The residues involved in pan DR binding ability are as described above; namely, X_0 is an amino acid selected from the group consisting of K and A; X_1 is an amino acid selected from the group consisting of (X), Y and F; X_2 is an amino acid selected from the group consisting of I and V; and X_6 is T. To obtain an stimulatory peptide, at least one of residues X_3, X_4, X_5, X_7, X_8, and X_9 is a polar, charged, aromatic, and/or bulky amino acid. In presently preferred embodiments, X_0 is also a polar, charged, aromatic, and/or bulky amino acid. *See* Table 4 for a listing of amino acids that are preferred at the particular positions. The non-polar, non-charged, non-aromatic, non-bulky amino acids used at the specified positions include, for example, tryptophan or lysine. In a presently preferred embodiment, an tryptophan is used at these positions. In additional preferred embodiments, X_7 or X_8, or both, are K.

Once a T cell stimulatory peptide is designed using the methods of the invention, it is desirable to test the peptide to ensure that it exhibits pan DR binding ability. Those peptides that do exhibit such binding ability are then tested for biological activity. For example, the peptides can be tested for ability to induce T cell activation in an *in vitro* assay system. Induction of T cell activation is a reliable indicator of a peptide's ability to induce T cell helper capacity. Suitable *in vitro* assays for T cell activation, *e.g.*, T cell proliferation and lymphokine secretion assays, are known to those of skill in the art.

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Table 3

pan-DR Binding Core	N-terminal (Z_n)	1	2	3	4	5	6	7	8	9	C-terminal (Z_c)
pan-DR Binding Core	Pref. at least 1 amino acid for stability; preferably at least one D amino acid	(X), Y, F, M, L, I, V or W	I, V	Any	Any	Any	T, V, M, S, A, C, P, L, or I	Any	Any	Any	Pref. at least 1 amino acid for stability; preferably at least one D amino acid
Inhibitory-- same as core plus:	A[1]			A[1]	A[1]	A[1]		A[1]	A[1]	A[1]	A[1]
Stimulatory-- same as core plus:	W[2]			W[2]	W[2]	W[2]		W[2]	W[2]	W[2]	W[2]

Table 4

pan-DR Binding Core	N-terminal (Z_n)	0	1	2	3	4	5	6	7	8	9	C-terminal (Z_c)
pan-DR Binding Core	Pref. at least 1 amino acid for stability; preferably at least one D amino acid	K, A	(X), Y, F	I, V	Any	Any	Any	T	Any	Any	Any	Pref. at least 1 amino acid for stability; preferably at least one D amino acid
Inhibitory-- same as core plus:	A[1]	A[1]			A[1]	A[1]	A[1]		A[1]	A[1]	A[1]	A[1]
Stimulatory-- same as core plus:	W[2]	W[2]			W[2]	W[2]	W[2]		W[2] (esp. K)	W[2] (esp. K)	W[2]	W[2]

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[1] Or other non-polar, non-charged, non-bulky, non-aromatic amino acid

[2] Or other polar, charged, bulky, or aromatic amino acid

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Examples of pan DR binding peptides of the invention that can induce or enhance a T-helper cell mediated immune response include, for example, the first 8 peptides listed in Table 9. This Table provides an illustration of various substitutions that one can make to obtain different pan DR stimulatory peptides. For example, the peptide 965.10 is a

5   synthetic peptide, having a non-naturally occurring cyclohexylalanine or similar peptide at position X_2 and being flanked on each end by D-amino acids. An analogous preferred peptide has a substitution, e.g., phenylalanine, at position X_2 of peptide 965.10. To obtain an all-natural yet analogous peptide, the D-amino acids at each end can be replaced by L-amino acids in addition to the substitution of a naturally occurring amino acid for the

10   cyclohexylalanine; an all-L-amino acid peptide such as this can be prepared and/or administered using nucleic acids that encode the peptide. Each of these three peptides can then be subjected to an additional substitution at position X_6, as illustrated in Table 5. For example, the tryptophan at position X_6 of peptide 965.10 or its two derivatives can be replaced by asparagine, tyrosine, lysine, histidine, or alanine without loss of stimulatory

15   activity. Thus, preferred peptides include those shown in Table 5.

Table 5

Amino acid at Position X_6	Synthetic	Replacement of Cyclohexylalanine	All-Natural (no D-amino acids or cyclohexylalanine)
W	aK(X)VAAWTLKAAa (SEQ ID NO:11)	aKFVAAWTLKAAa (SEQ ID NO:12)	AKFVAAWTLKAAA (SEQ ID NO:13)
N	aK(X)VAANTLKAAa (SEQ ID NO:14)	aKFVAANTLKAAa (SEQ ID NO:15)	AKFVAANTLKAAA (SEQ ID NO:16)
Y	aK(X)VAAYTLKAAa (SEQ ID NO:17)	aKFVAAYTLKAAa (SEQ ID NO:18)	AKFVAAYTLKAAA (SEQ ID NO:19)
K	aK(X)VAAKTLKAAa (SEQ ID NO:20)	aKFVAAKTLKAAa (SEQ ID NO:21)	AKFVAAKTLKAAA (SEQ ID NO:22)
H	aK(X)VAAHTLKAAa (SEQ ID NO:23)	aKFVAAHTLKAAa (SEQ ID NO:24)	AKFVAAHTLKAAA (SEQ ID NO:25)
A	aK(X)VAAATLKAAa (SEQ ID NO:26)	aKFVAAATLKAAa (SEQ ID NO:27)	AKFVAAATLKAAA (SEQ ID NO:28)

B.   *Computer Screening of Protein Sequences for Pan DR Binding Peptides*

Computer programs that allow the rapid screening of protein sequences for the occurrence of the subject pan DR binding motifs are encompassed by the present

20   invention. These programs are implemented to analyze any identified amino acid sequence, or to operate on an unknown sequence and simultaneously determine the sequence and

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identify motif-bearing epitopes thereof. Generally, the identified sequences will be from a pathogenic organism or a tumor-associated peptide.

In utilizing computer screening to identify peptide epitopes, a protein sequence or translated sequence may be analyzed using software developed to search for motifs, for example the "FINDPATTERNS' program (Devereux, *et al. Nucl. Acids Res.* 12:387-395, 1984) or MotifSearch 1.4 text string software program (D. Brown, San Diego, CA) to identify potential peptide sequences containing a pan DR binding motif.

As noted above, the pan DR motif includes the presence of particular amino acid residues, as set forth in Tables 3 and 4, at core positions X_1, X_2, and X_6. In certain embodiments, the motif may be further refined to identify those peptides that bear stimulatory or inhibitory residues, as defined in Tables 3 and 4, at particular positions of the peptide epitope.

As appreciated by one of ordinary skill in the art, a large array of computer programming software and hardware options are available in the relevant art which can be employed to implement the motifs of the invention in order to evaluate (*e.g.*, without limitation, to identify epitopes, identify epitope concentration per peptide length, or to generate analogs) known or unknown peptide sequences. Furthermore, such calculations can be made mentally.

Once peptide sequences that bear pan-DR binding motifs have been identified, the corresponding peptides can be synthesized and tested to confirm the ability to bind multiple DR molecules. Those peptides that exhibit pan DR binding activity, *i.e.*, peptides that bind to multiple, preferably at least three, four, five or six allele-specific DR molecules, may then be tested for the ability to stimulate HTL activity using *in vitro* assays, *e.g.* T cell proliferation (*see, e.g.* Alexander *et al., J. Immunol.* 159:4753-4761, 1997) known by those in the art.

Pan DR motif-bearing peptides identified as described above may further be modified to exhibit enhanced T cell stimulating activity or inhibitory T cell activity. Such modifications may be made using the inhibitory or stimulatory residues identified in Tables 3 and 4 as guidance. The modified peptides may then be tested for increased T cell stimulating or inhibitory activity.

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C. *Use of Pan DR Peptides to Modulate Immune Responses*

The invention also provides methods and compositions in which pan DR peptides are used for modulating immune responses. The particular peptide employed determines whether the modulation is inhibition or stimulation.

1. *Inhibition of T cell-mediated immune responses*

In one embodiment, the present invention provides methods of using the inhibitory pan DR peptides to block an immune response by preventing activation of helper T cells. As a result of their cross-reactive class II binding capacity, the pan DR binding peptides can be used as therapeutics in the inhibition of T cell mediated events involved in allograft rejection, allergic responses, autoimmunity, and the like. The administration of the inhibitory pan DR peptides of the invention to an animal in need of treatment for such conditions and subsequent binding of the peptide to the MHC molecules can block the ability of the MHC to bind peptides that are involved in eliciting or exacerbating the inappropriate immune reaction.

The pan DR binding peptides can be used to treat a variety of conditions involving unwanted T cell reactivity. Examples of diseases which can be treated using pan DR binding peptides include autoimmune diseases (*e.g.*, rheumatoid arthritis, multiple sclerosis, and myasthenia gravis), allograft rejection, allergies (*e.g.*, pollen or pet allergies), Lyme disease, hepatitis, LCMV, post-streptococcal endocarditis, or glomerulonephritis, and food hypersensitivities.

2. *Stimulation of helper T cell mediated immune responses*

Particularly preferred embodiments provided by the invention are methods of using the stimulatory pan DR peptides, optionally with an adjuvant component in any vaccine formulation to enhance an immune response against an administered immunogen. For example, the pan DR binding peptides can be admixed with or conjugated with a CTL-inducing peptide and administered to induce a CTL response against, *e.g.*, virally infected or tumor-associated antigen-bearing cells. In another embodiment, the pan DR peptides are conjugated with antibody-inducing peptides or carbohydrates. In addition, the pan DR peptides can be admixed with an antibody-inducing peptide or carbohydrate. The use of helper peptides to enhance antibody responses against particular determinants is described in, *e.g.*, Hervas-Stubbs *et al.*, *Vaccine* 12:867-871 (1994).

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The one or more CTL and/or antibody-inducing peptides or other antigens can be administered with one or more pan DR peptides in a mixture that may or may not involve noncovalent associations between the peptides and/or other antigens. For instance, one or more of the peptides may be lipidated.

5   Alternatively, the peptides can be covalently linked to form a PADRE-antigenic determinant. To facilitate the association of the antigenic determinant with the PADRE peptide, additional amino acids can be added to the termini of the peptides. The additional residues can also be used for coupling to a carrier, support or larger peptide, for reasons discussed herein, or for modifying the physical or chemical properties of the

10   peptide or oligopeptide, or the like. Amino acids such as tyrosine, cysteine, lysine, glutamic or aspartic acid, or the like, can be introduced at the C- or N-terminus of the peptide or oligopeptide. In addition, the peptide or oligopeptide sequences can differ from the natural sequence by being modified by terminal-NH_2 acylation, e.g., by alkanoyl (C_1-C_{20}) or thioglycolyl acetylation, terminal-carboxy amidation, e.g., ammonia, methylamine, etc. In

15   some instances these modifications may provide sites for linking to a support or other molecule.

Accordingly, the CTL- or antibody-inducing peptide, carbohydrate, or other antigen can be linked to the pan DR binding peptide either directly or via a spacer either at the amino or carboxy terminus of the CTL peptide. The amino terminus of either the CTL-

20   or antibody-inducing peptide or the pan DR binding peptide may be acylated to facilitate linkage. For example, the conjugate of a CTL or antibody-inducing antigen which is linked to the pan DR peptide can in turn be linked to certain alkanoyl (C_1-C_{20}) lipids via one or more linking residues such as Gly, Gly-Gly, Ser, Ser-Ser as described below.

In particularly preferred embodiments, CTL- or antibody-inducing peptides or

25   carbohydrate epitope/pan DR binding conjugates of the invention are linked by a spacer molecule. See, e.g., copending US patent application No. 08/788,822, filed January 23, 1997. Alternatively, the peptide or other antigen can be linked to the pan DR binding peptide without a spacer. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under

30   physiological conditions and may have linear or branched side chains, and also natural amino (butyric, caproic, hexanoic, octanoic, lauric, or palmitic) acid. The spacers are

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typically selected from, *e.g.*, Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. In certain preferred embodiments herein the neutral spacer is Ala. It will be understood that the optionally present spacer need not be comprised of the same residues and thus may be a hetero- or homo-oligomer. Preferred exemplary spacers are

5   homo-oligomers of Ala and amino caproic acid. When present, the spacer will usually be at least one or two residues, more usually three to 16 residues. In other embodiments the pan DR binding peptide is conjugated to the CTL- or antibody-inducing peptide, carbohydrate, or other antigen, preferably with the pan DR binding peptide positioned at the amino terminus. The two moieties can be joined by a neutral linker, such as Ala-Ala-Ala or the like.

10   Moreover, a pan DR binding peptide can be linked to another molecule by means of a spacer which is a surface active molecule such as a lipid or surfactant.

Where the same peptide is linked to itself, thereby forming a homopolymer, a plurality of repeating epitopic units is presented. For example, multiple antigen peptide (MAP) technology can be used to construct polymers containing both CTL and/or antibody

15   peptides and PADRE peptides. When the peptides differ, *e.g.*, a cocktail representing different viral subtypes, different epitopes within a subtype, different HLA restriction specificities, or peptides which contain T helper epitopes, heteropolymers with repeating units are provided. In addition to covalent linkages, noncovalent linkages capable of forming intermolecular and intrastructural bonds are also contemplated.

20   In some embodiments it may be desirable to include in the pharmaceutical compositions of the invention at least one component which assists in priming CTL. For example, one or more surface-active molecules can be included in the compositions. These compounds include, for example, lipids, polymers (*e.g.*, nonionic block copolymers), polyalkylene glycol (*e.g.*, polyethylene glycol) and surfactants. The inclusion in the

25   compositions of such compounds, which can cause the peptides to become membrane-bound, can alter how the peptide is delivered to cells or tissues and can also affect clearance and/or degradation.

Lipids are one example of components that have been identified as agents capable of assisting the priming of CTL *in vivo* against viral antigens. For example, palmitic

30   acid residues can be attached to the alpha and epsilon amino groups of a Lys residue and then linked, *e.g.*, via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the

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like, to an immunogenic peptide. The lipidated peptide can then be injected directly in a micellar form, incorporated into a liposome or emulsified in an adjuvant, *e.g.*, incomplete Freund's adjuvant. In a preferred embodiment a particularly effective immunogen comprises palmitic acid attached to alpha and epsilon amino groups of Lys, which is attached via

5   linkage, *e.g.*, Ser-Ser, to the amino terminus of the immunogenic peptide.

As another example of lipid priming of CTL responses, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinlyseryl-serine (P_3CSS) can be used to prime virus specific CTL when covalently attached to an appropriate peptide. *See*, Deres *et al.*, *Nature* 342, 561-564 (1989). Peptides of the invention can be coupled to P_3CSS, for example, and

10   the lipopeptide administered to an individual to specifically prime a CTL response to the target antigen. Further, as the induction of neutralizing antibodies can also be primed with P_3CSS conjugated to a peptide which displays an appropriate epitope, the two compositions can be combined to more effectively elicit both humoral and cell-mediated responses to an infection or tumor.

15   The antibody-inducing or CTL-inducing antigen (*e.g.*, a polysaccharide, protein, glycoprotein, lipid, glycolipid, lipopolysaccharide, and the like) that is administered in conjunction with the pan DR peptides of the invention can be an antigen that is associated with, e.g., a pathogen, a diseased cell, etc. For example, the antigen can be from a bacterium, a virus, a cancer cell, a fungus, or a parasite, among others. Peptide and carbohydrate

20   epitopes from a large number of antigenic biomolecules can also be used in the conjugates and compositions of the present invention. For a listing of suitable antigens for use in the present invention, *see, e.g.*, BioCarb Chemicals Catalogue; and *The Jordan Report: Accelerated Development of Vaccine 1995* NIH, Bethesda, Maryland, 1995). Some examples of suitable antigens are described below.

25   a. CTL- and antibody-inducing peptides.

CTL and/or antibody-inducing peptides can be administered with the pan DR peptides of the invention to enhance an immune response. CTL and antibody epitopes from a number of antigenic proteins can be used in the conjugates of the present invention. Examples of suitable antigens include prostate specific antigen (PSA), hepatitis B core and

30   surface antigens (HBVc, HBVs), hepatitis C antigens, Epstein-Barr virus antigens, human immunodeficiency virus (HIV) antigens and human papilloma virus (HPV) antigens. An

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exemplary viral antigen includes those derived from HIV (*e.g.*, gp120). Exemplary fungal antigens include those derived from *Candida albicans, Cryptococcus neoformans, Coccidoides spp., Histoplasma spp., and Aspergillus spp.* Parasitic antigens include those derived from *Plasmodium spp., trypanosoma spp., Schistosoma spp., Leishmania spp.*, and

5 the like. Additionally, examples of tumor-associated antigens include carcinoembryonic antigen, p53, melanoma antigens (*e.g.*, MAGE-1, -2, or -3), HER2/neu, or prostate-related antigens (e.g., PSA).

In certain embodiments the CTL peptides of the invention are derived from within the HBV surface antigen or the nucleocapsid polypeptides, core and precore. In more

10 preferred embodiments described herein the CTL-inducing peptides are derived from the region of HBenv$_{309-328}$ (peptide 799.08), HBenv$_{329-348}$ (peptide 799.09), HBenv$_{349-368}$ (peptide 799.10), or the region HBc$_{91-110}$ (peptide 802.03), where the numbering is according to Galibert *et al., Nature* 281, 646 (1979).

CTL- and antibody-inducing peptides, and how such peptides are obtained,

15 are described in more detail in US Patent Application No. 08/485,218, filed June 7, 1995.

b. CTL- and antibody-inducing carbohydrate epitopes.

Carbohydrate epitopes, both antibody-inducing and CTL-inducing, from a wide variety of sources can be used in conjunction with the pan DR peptides of the invention. For example, carbohydrate epitopes associated with many types of cancer, or

20 infectious agents (*e.g.*, bacteria, fungi, parasites, viruses, and the like), are described in more detail in U.S. Patent Application No. 08/788,822, filed July 23, 1997.

Examples of suitable antigens include those derived from bacterial surface polysaccharides and which can be used in carbohydrate-based vaccines. Bacteria typically express carbohydrate epitopes on their cell surface, including glycoproteins, glycoplipids,

25 O-specific side chains of lipopolysaccharides, capsular polysaccharides and the like. Exemplary bacterial strains include *Streptococcus pneumonia, Neisseria meninitidis, Haemophilus influenza, Klebsiella spp., Pseudomonas spp., Salmonella spp., Shigella spp.*, and Group B *streptococci.* A number of suitable bacterial carbohydrate epitopes are described in the art *(see, e.g.,* Sanders *et al. Pediatr. Res.* 37:812-819 (1995); Bartoloni *et al.*

30 *Vaccine* 13:463-470 (1995); Pirofski *et al., Infect. Immun.* 63:2906-2911 (1995) and International Publication No. WO 93/21948). Examples of suitable tumor carbohydrate

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antigens include GM2, GD2, GD3, Globo H, Le^y, Sialyl Le^a, T epitope, T$_N$ epitope, ST$_N$ epitope as described in the art (*see, e.g.*, Livingston *et al., Cancer Immunol. Immununother.* 45:1-9 (1997)).

D.   *Pharmaceutical Compositions*

The compounds of the present invention, and pharmaceutical and vaccine compositions thereof, can be administered to mammals, particularly humans, for prophylactic and/or therapeutic purposes. In preferred embodiments, the present invention is used to elicit and/or enhance immune responses against immunogens. For instance, CTL peptide-pan DR peptide mixtures may be used to treat and/or prevent viral infection or cancer. Alternatively, carbohydrate immunogens can be used. Examples of diseases which can be treated using the present invention include various bacterial infections, viral infections, fungal infections, parasitic infections and cancer. In other embodiments, inhibitory pan DR peptides of the invention are administered to block an inappropriate or otherwise undesirable immune response (*e.g.*, autoimmunity and the like).

In therapeutic applications, the present invention is administered to an individual already suffering from an inappropriate immune response, cancer, or infected with the pathogen of interest. Those in the incubation phase or the acute phase of the disease can be treated with the present invention separately or in conjunction with other treatments, as appropriate.

In therapeutic applications, a composition of the present invention is administered to a patient in an amount sufficient to block an undesired immune response, or to elicit an effective CTL response or humoral response to the microorganism or tumor antigen and to cure or at least partially arrest symptoms and/or complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, *e.g.*, the peptide composition, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician.

Therapeutically effective amounts of the compositions of the present invention generally range for the initial immunization that is for therapeutic or prophylactic administration, from about 1.0 µg to about 10,000 µg of peptide for a 70 kg patient, usually

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from about 100 to about 8000 µg, and preferably between about 200 and about 6000 µg. These doses are followed by boosting dosages of from about 1.0 µg to about 1000 µg of peptide pursuant to a boosting regimen over weeks to months depending upon the patient's response and condition as determined by measuring specific immune responses.

5   It must be kept in mind that the compositions of the present invention may generally be employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, in view of the minimization of extraneous substances and the relative nontoxic nature of the conjugates, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these compositions.

10   Further, the present invention can be used prophylactically to prevent and/or ameliorate inappropriate immune responses, bacterial infections, viral infections, fungal infections, parasitic infections or cancer. Effective amounts are as described above. Additionally, one of ordinary skill in the vaccine arts understands how to adjust or modify prophylactic treatments, as appropriate, for example by boosting and adjusting dosages and 15   dosing regimes in accordance with known immunology and pharmacology principles.

Therapeutic administration may begin at the first sign of disease or the detection or surgical removal of tumors or shortly after diagnosis in the case of acute infection. This is followed by boosting doses until at least symptoms are substantially abated and for a period thereafter. In chronic infection, loading doses followed by boosting doses 20   may be required.

Treatment of an infected individual with the compositions of the invention may hasten resolution of the infection in acutely infected individuals. For those individuals susceptible (or predisposed) to developing chronic infection the compositions are particularly useful in methods for preventing the evolution from acute to chronic infection. 25   Where the susceptible individuals are identified prior to or during infection, the composition can be targeted to them, minimizing need for administration to a larger population.

The present invention is also be used for the treatment of chronic infection and to stimulate the immune system to eliminate, e.g., virus-infected cells in carriers. It is important to provide compositions of the present invention in an amount, in a formulation 30   and via a mode of administration sufficient to effectively elicit and/or enhance an immune response. Thus, for treatment of chronic infection, a representative dose is in the range of

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about 1.0 µg to about 5000 µg, preferably about 5 µg to 1000 µg for a 70 kg patient per dose. Immunizing doses followed by boosting doses at established intervals, *e.g.*, from one to four weeks, may be required, possibly for a prolonged period of time to effectively immunize an individual. In the case of chronic infection, administration should continue

5   until at least clinical symptoms or laboratory tests indicate that the viral infection has been eliminated or substantially abated and for a period thereafter.

The pharmaceutical compositions for therapeutic or prophylactic treatment are intended for parenteral, topical, oral or local administration. Typically, the pharmaceutical compositions are administered parenterally, *e.g.*, intravenously,

10   subcutaneously, intradermally, or intramuscularly. The vaccine compositions of the invention are particularly suitable for oral administration, due to their ease of administration. Thus, the invention provides compositions for parenteral administration which comprise a solution of the peptides or conjugates dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, *e.g.*, water,

15   buffered water, 0.9% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to

20   approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate and the like.

Carriers can be used in combination with peptides of the invention. Carriers

25   are well known in the art, and include, *e.g.*, thyroglobulin, albumins such as bovine serum albumin, tetanus toxoid, polyamino acids such as poly(lysine:glutamic acid), hepatitis B virus core protein, hepatitis B virus recombinant vaccine and the like. The vaccines can also contain a physiologically tolerable (acceptable) diluent such as water, phosphate buffered saline, or saline, and further typically include an adjuvant. Adjuvants such as incomplete

30   Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are materials well known in the art.

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The concentration of compositions of the present invention in the pharmaceutical formulations can vary widely, *i.e.*, from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

The present invention may also be administered via liposomes, which serve to target the conjugates to a particular tissue, such as lymphoid tissue, or targeted selectively to infected cells, as well as increase the half-life of the peptide composition. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations the composition to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to, *e.g.*, a receptor prevalent among lymphoid cells, such as monoclonal antibodies which bind to the CD45 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes filled with a desired composition of the present invention can be directed to the site of lymphoid cells, where the liposomes then deliver the selected therapeutic/immunogenic peptide compositions. Liposomes for use in the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, *e.g.*, liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, *e.g.*, Szoka *et al.*, *Ann. Rev. Bioshys. Bioeng.* 9, 467 (1980), U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

For targeting to the immune cells, a ligand to be incorporated into the liposome can include, *e.g.*, antibodies or fragments thereof specific for cell surface determinants of the desired immune system cells. A liposome suspension containing a composition of the present invention may be administered intravenously, locally, topically, etc. in a dose which varies according to, *inter alia,* the manner of administration, the composition being delivered, and the stage of the disease being treated.

Alternatively, DNA or RNA encoding one or more PADRE peptides and a polypeptide containing one or more CTL epitopes or antibody-inducing epitopes may be introduced into patients to obtain an immune response to the polypeptides which the nucleic

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acid encodes. Wolff *et. al., Science* 247: 1465-1468 (1990) describes the use of nucleic acids to produce expression of the genes which the nucleic acids encode.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more conjugates of the invention, and more preferably at a concentration of 25%-75%.

For aerosol administration, the compositions of the present invention are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of the composition are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, *e.g.,* lecithin for intranasal delivery.

As noted herein, compositions of the invention may be introduced into a host, including humans, linked to its own carrier or as a homopolymer or heteropolymer of active peptide units. Such a polymer has the advantage of increased immunological reaction and, where different peptides are used to make up a hetero polymer, the ability to induce antibodies and/or CTLs that react with different antigenic determinants.

And, as mentioned above, immune responses can be primed by conjugating compositions of the present invention to lipids, such as P$_3$CSS. Upon immunization with a composition as described herein, via injection, aerosol, oral, transdermal or other route, the immune system of the host responds by producing an enhanced immune response, humoral and/or cellular.

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In some instances it may be desirable to combine the compositions of the present invention with other modalities, such as vaccines which induce neutralizing antibody responses to infections and cancers of interest.

The peptides of this invention can also be used to make monoclonal antibodies. Such antibodies are useful as diagnostic or therapeutic agents.

The peptides of the invention are also used as diagnostic reagents. For example, a peptide of the invention can be used to determine the susceptibility of a particular individual to a treatment regimen which employs the peptide or related peptides, and thus can be helpful in modifying an existing treatment protocol or in determining a prognosis for an affected individual. In addition, the peptides can also be used to predict which individuals will be at substantial risk for developing chronic infection.

E. Construction of Expression Vectors for Nucleic Acids Encoding Pan DR Binding Peptides and Administration In Vivo

The peptides of the invention can be delivered by way of nucleic acids ("minigenes") through the construction of expression vectors encoding a peptide epitope of interest. Such vectors will contain at least one promoter element that is capable of expressing a transcription unit encoding at least one pan DR binding peptide and an MHC targeting sequence for the appropriate cells of an organism, so that the antigen is expressed and targeted to the appropriate MHC molecule. For example, if the expression vector is administered to a mammal such as a human, a promoter element that functions in a human cell is incorporated into the expression vector. The vectors may also optimally include nucleic acid sequences encoding multiple pan DR binding peptides and/or one or more MHC class I epitopes.

Routine techniques in the field of recombinant genetics may be used to construct the expression vectors. Basic texts disclosing the general methods of use in this invention include Sambrook *et al., Molecular Cloning, A Laboratory Manual* (2nd ed. 1989); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds., 1994); *Oligonucleotide Synthesis: A Practical Approach* (Gait, ed., 1984); Kuijpers, *Nucleic Acids Research* 18(17):5197 (1994); Dueholm, *J. Org. Chem.* 59:5767-5773 (1994); *Methods in Molecular Biology*, volume 20 (Agrawal, ed.); and Tijssen, *Laboratory Techniques in Biochemistry and Molecular Biology-*

-Hybridization with Nucleic Acid Probes, e.g., Part I, chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays" (1993)).

The minigenes can comprise one or many different epitopes. The nucleic acid encoding the epitopes are assembled in a minigene according to standard techniques. In general, the nucleic acid sequences encoding minigene epitopes are isolated using amplification techniques with oligonucleotide primers, or are chemically synthesized. Recombinant cloning techniques can also be used when appropriate. Oligonucleotide sequences are selected which either amplify (when using PCR to assemble the minigene) or encode (when using synthetic oligonucleotides to assemble the minigene) the desired epitopes.

Amplification techniques using primers are typically used to amplify and isolate sequences encoding the epitopes of choice from DNA or RNA (*see* U.S. Patents 4,683,195 and 4,683,202; *PCR Protocols: A Guide to Methods and Applications* (Innis *et al.*, eds, 1990)). Methods such as polymerase chain reaction (PCR) and ligase chain reaction (LCR) can be used to amplify epitope nucleic acid sequences directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. Restriction endonuclease sites can be incorporated into the primers. Minigenes amplified by the PCR reaction can be purified from agarose gels and cloned into an appropriate vector.

Synthetic oligonucleotides can also be used to construct minigenes. This method is performed using a series of overlapping oligonucleotides, representing both the sense and non-sense strands of the gene. These DNA fragments are then annealed, ligated and cloned. Oligonucleotides that are not commercially available can be chemically synthesized according to the solid phase phosphoramidite triester method first described by Beaucage & Caruthers, *Tetrahedron Letts*. 22:1859-1862 (1981), using an automated synthesizer, as described in Van Devanter *et. al., Nucleic Acids Res*. 12:6159-6168 (1984). Purification of oligonucleotides is by either native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson & Reanier, *J. Chrom*. 255:137-149 (1983).

The epitopes of the minigene are typically subcloned into an expression vector that contains a strong promoter to direct transcription, as well as other regulatory sequences such as enhancers and polyadenylation sites. Suitable promoters are well known in the art and described, *e.g.*, in Sambrook *et al*. and Ausubel *et al*. Eukaryotic expression

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systems for mammalian cells are well known in the art and are commercially available. Such promoter elements include, for example, cytomegalovirus (CMV), Rous sarcoma virus LTR and SV40.

The expression vector typically contains a transcription unit or expression

5   cassette that contains all the additional elements required for the expression of the minigene in host cells. A typical expression cassette thus contains a promoter operably linked to the minigene and signals required for efficient polyadenylation of the transcript. Additional elements of the cassette may include enhancers and introns with functional splice donor and acceptor sites.

10   In addition to a promoter sequence, the expression cassette can also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes.

The particular expression vector used to transport the genetic information into

15   the cell is not particularly critical. Any of the conventional vectors used for expression in eukaryotic cells may be used. Expression vectors containing regulatory elements from eukaryotic viruses are typically used in eukaryotic expression vectors, e.g., SV40 vectors, papilloma virus vectors, and vectors derived from Epstein Barr virus. Other exemplary eukaryotic vectors include pMSG, pAV009/A+, pMTO10/A+, pMAMneo-5, baculovirus

20   pDSVE, and any other vector allowing expression of proteins under the direction of the SV40 early promoter, SV40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells. In one embodiment, the vector pEP2 is used in the present invention.

25   Other elements that are typically included in expression vectors also include a replicon that functions in *E. coli*, a gene encoding antibiotic resistance to permit selection of bacteria that harbor recombinant plasmids, and unique restriction sites in nonessential regions of the plasmid to allow insertion of eukaryotic sequences. The particular antibiotic resistance gene chosen is not critical, any of the many resistance genes known in the art are

30   suitable. The prokaryotic sequences are preferably chosen such that they do not interfere with the replication of the DNA in eukaryotic cells, if necessary.

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The invention further relates to methods of administering a pharmaceutical composition comprising an expression vector of the invention to stimulate an immune response. The expression vectors are administered by methods well known in the art as described in Donnelly *et al.* (*Ann. Rev. Immunol.* 15:617-648 (1997)); Felgner *et al.* (U.S. Patent No. 5,580,859, issued December 3, 1996); Felgner (U.S. Patent No.5,703,055, issued December 30, 1997); and Carson *et al.* (U.S. Patent No. 5,679,647, issued October 21, 1997).

A pharmaceutical composition comprising an expression vector of the invention can be administered to stimulate an immune response in a subject by various routes including, for example, orally, intravaginally, rectally, or parenterally, such as intravenously, intramuscularly, subcutaneously, intraorbitally, intracapsularly, intrathecally, intraperitoneally, intracisternally or by passive or facilitated absorption through the skin using, for example, a skin patch or transdermal iontophoresis, respectively. Furthermore, the composition can be administered by injection, intubation or topically, the latter of which can be passive, for example, by direct application of an ointment or powder, or active, for example, using a nasal spray or inhalant. An expression vector also can be administered as a topical spray, in which case one component of the composition is an appropriate propellant. The pharmaceutical composition also can be incorporated, if desired, into liposomes, microspheres or other polymer matrices (Felgner *et al.*, U.S. Patent No. 5,703,055; Gregoriadis, *Liposome Technology*, Vols. I to III (2nd ed. 1993), each of which is incorporated herein by reference). Liposomes, for example, which consist of phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

The expression vectors of the invention can be delivered to the interstitial spaces of tissues of an animal body (Felgner *et al.*, U.S. Patent Nos. 5,580,859 and 5,703,055). Administration of expression vectors of the invention to muscle is a particularly effective method of administration, including intradermal and subcutaneous injections and transdermal administration. Transdermal administration, such as by iontophoresis, is also an effective method to deliver expression vectors of the invention to muscle. Epidermal administration of expression vectors of the invention can also be employed. Epidermal administration typically involves mechanically or chemically irritating the outermost layer of

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epidermis to stimulate an immune response to the irritant (Carson *et al.*, U.S. Patent No. 5,679,647).

Other effective methods of administering an expression vector of the invention to stimulate an immune response include mucosal administration (Carson *et al.*,

5   U.S. Patent No. 5,679,647). For mucosal administration, a presently preferred method of administration comprises intranasal administration of an appropriate aerosol containing the expression vector and a pharmaceutical composition. Suppositories and topical preparations are also effective for delivery of expression vectors to mucosal tissues of genital, vaginal and ocular sites. Additionally, expression vectors can be complexed to particles and

10   administered by a vaccine gun.

The dosage of a minigene construct to be administered is dependent on the method of administration and will generally be between about 0.1 μg up to about 200 μg. For example, the dosage can be from about 0.05 μg/kg to about 50 mg/kg, in particular about 0.005-5 mg/kg. An effective dose can be determined, for example, by measuring the

15   immune response after administration of an expression vector. For example, the production of antibodies specific for the MHC class II epitopes or MHC class I epitopes encoded by the expression vector can be measured by methods well known in the art, including ELISA or other immunological assays. In addition, the activation of T helper cells or a CTL response can be measured by methods well known in the art including, for example, the uptake of ^{3}H-

20   thymidine to measure T cell activation and the release of ^{51}Cr to measure CTL activity.

EXAMPLES

The following examples are offered to illustrate, but not to limit the present invention.

<center>**Example 1**</center>

<center>**Pan DR Binding Sequence provides T-cell Help for Induction of Protective Antibodies against *Plasmodium yoelli* sporozoites**</center>

1.   Introduction

5   Pan DR epitopes (PADRE) as disclosed herein bind to most common HLA DR molecules. The pan DR peptides are immunogenic for human T cells, as described herein and in Alexander *et al., Immunity* **1**:751-61 (1994). PADRE peptides fortuitously also bind the mouse MHC molecules, IA^b, thus providing a test system for demonstrating that that they deliver powerful helper T-cell activity *in vivo*, as demonstrated by

10   enhancement of specific responses directed against an influenza virus-derived class I-restricted epitope.

The capacity of PADRE epitopes to deliver help for antibody responses *in vivo* is also described herein, and has been subsequently published (Del Guercio *et al., Vaccine* **15**:441-8 (1997)). According to a commonly held view, efficient induction of

15   antibody responses requires large multivalent antigens, which can fulfill the requirements for cross-linking of surface Ig on the surface of specific B-cells. Consequently it was assumed that single, monovalent antigens are not efficient immunogens for B-cell responses. However, the peptides of the present invention, which include short, linear, monovalent constructs that contain the PADRE epitope, are remarkably active immunogens *in vivo*, in

20   terms of induction of specific antibody responses.

In one demonstration of this antibody-inducing activity, the *Plasmodium vivax* circumsporozoite protein B-cell epitope (PvB), previously shown to be exquisitely T-cell dependent (Nardin *et al., Eur J. Immunol* **17**:1763-7 (1987)), was incorporated in 33-residue long peptide immunogen. This construct induced responses almost exclusively

25   composed of IgG, long lasting and indistinguishable in titer from those induced by more complex multivalent branched chain polymer multiple antigenic peptide (MAP) and KLH conjugates (Del Guercio *et al., Vaccine* **15**:441-8 (1997)). The efficacy of this type of single monovalent construct was generalized to B-cell epitopes derived from other parasites (*P. yoelii* and *P. falciparum*). Resulting antibodies were shown to bind intact sporozoites, thus

<center>40</center>

suggesting that these responses were of potential biological relevance (Del Guercio, M.F. *et al., Vaccine* **15**:441-8 (1997)).

This Example demonstrates that the antibodies induced by the *P. falciparum* and *P. yoelii* CSP-PADRE peptides inhibit sporozoite invasion of hepatocytes *in vitro*, and that immunization with the PyCSP-PADRE linear peptide protects mice against challenge with *P. yoelii* sporozoites comparably to the PyCSP-MAP (Franke *et al., Vaccine* 17:1201-1205 (1999)).

2.   Methods

2.1.   Mice

Four to six week old female C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME).

2.2.   *Immunogens*

2.2.1   *Linear peptides*

Peptides encompassing B-cell epitopes from the central immunodominant circumsporozoite repeat region of circumsporozoite proteins (CSP) of *P. yoelii* (PyB) or *P. falciparum* (PfB) were synthesized by standard MOC chemistry, purified by HPLC and their purity and identity verified by HPLC and mass spectrometry. Sequences: PyB = G(QGPGAP)$_4$ (Charoenvit, Y. *et al., J. Immunol.* **146**:1020-5 (1991)); PfB = (NANP)$_4$ (Nussenzweig, V. *et al., Adv Immunol* **45**:283-334 (1989); Dame, J.B. *et al., Science* **225**:593-9 (1984)). Peptides colinearly synthesized to encompass PADRE were also produced using the same methods. PADRE-PfB sequence: aKXVAAWTLKAa(NANP)$_4$GGS; PADRE-PyB sequence: aKXVAAWTLKAa(QGPGAP)$_4$GGS.

2.2.2.   *PyCSP-MAP*

A multiple antigen peptide (PyCSP-MAP) was also synthesized as previously described (Wang, R. *et al., J. Immunol* **154**:2784-93 (1995); Valmori, D. *et al., J Immunol Meth* **149**:717-21 (1992)). In brief, it included a lysine core and four branches. Each branch included four copies of the protective B-cell epitope, QGPGAP, from the PyCSP and the

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universal T-helper epitopes from tetanus toxin, p2p30 (p2 = QYIKANSKFIGITE; p30 = FNNFTVSFWLRVPKVSASHLE) (Wang, R. *et al., J. Immunol* **154**:2784-93 (1995)).

2.3   Immunization

In certain experiments, peptides were emulsified in complete Freund's adjuvant (CFA) and injected subcutaneously (S.C.) at the base of the tail in 100 μl volume containing 100 μg of peptide. Mice were boosted with 100 μg of peptide emulsified in 100 μl of incomplete Freund's adjuvant (IFA) 1 month later. Mice were bled just prior to boosting, and 2 weeks after boosting.

In other experiments, the peptides were suspended in phosphate buffered saline (PBS) and emulsified in TiterMax™ (CytRx Corporation, Norcross, GA). Mice were immunized with peptides by S.C. injection at the base of the tail three times at 3-week intervals. The injection volume was 25 μl. The doses and regimens for these immunizations were based on those determined to be optimal from previous experiments (Del Guercio, M.F. *et al., Vaccine* **15**:441-8 (1997); Wang, R. *et al., J. Immunol* **154**:2784-93 (1995)) (Sette *et al.,* unpublished observations).

2.4   Cell culture

Mouse hepatocytes were obtained by *in situ* collagenase perfusion as previously described (Charoenvit, Y. *et al., Exp Parasitol* **80**:419-29 (1995)). Cells were plated at a concentraiton of 1 x 10^5 cells/well in 8-well chamberslides (Nunc, Naperville, IL) using Complete Medium (MEM with Earle's balanced salts, supplemented with 0.2% BSA, 10% fetal calf serum, 2% penicillin-streptomcyn, 2 mg insulin, 1% glutamine and 1% nonessential amino acids solution). All cell culture reagents were obtained from quality Biological, Gaithersburg, MD. The slides were incubated overnight at 37°C in a 5% CO_2/95% air environment. The medium was changed the following day and fresh medium containing dexamethasone (7 x 10^{-5}) was added to the cultures.

Human hepatocytes were isolated from nontransplantable liver tissue donated for research. A single cell suspension was made by a two-step collagenase perfusion as previously reported (Mellouk, S. *et al., Bull World Health Organ* **68 (Suppl)**:52-9 (1990)). The cells were seeded onto 8-well chamberslides (Nunc, Naperville, IL) at a concentration of

1.25 x 10^5 cells/well in Complete Medium. The hepatocytes were allowed to attach and spread overnight with the media being changed the following day.

2.4.1. Parasites

The 17XNL (nonlethal) strain of *P. yoelii* or the NF54 strain of *P. falciparum* were used for murine and human hepatocyte *in vitro* infections, respectively. Parasites were harvested using a modification of the Ozaki technique for the rapid isolation of sporozites (Ozaki, L.S. *et al., J. Parasitol* **70**:831-3 (1984)).

2.5. Inhibition of liver stage development assay (ILSDA)

The capacity of sera from immunized mice to inhibit sporozoite invasion and development in hepatocytes was assessed by ILSDA as previously described (Wang, R. *et al., J. Immunol* **154**:2784-93 (1995); Charoenvit, Y. *et al., J. Immunol.* **146**:1020-5 (1991)). Briefly, 25 μl of the serum samples, diluted to 2x their final concentration, were added to triplicate chamber-slide wells. *P. yoeilli* or *P. falciparum* sporozoites, 75,000/well, were then added in a volume of 25 μl to the mouse or human hepatocyte cultures respectively. The cultures were incubated for 3 h, then washed extensively with fresh medium to remove unattached sporozoites. The infected hepatocytes were then incubated for a further 2 (*P. yoelii*) or 5 days (*P. falciparum*) with the medium being changed each day of culture. The slides were fixed and immunostained with either a *P. yoelii-* or *P. falciparum*-specific monoclonal antibody. Schizonts were counted from the triplicate cultures and the percent inhibition determined as follows: control - test/control x 100. Sera from mice injected with CFA (first dose) and IFA (second and third doses) served as the negative controls.

2.6. Antibodies to sporozoites, synthetic peptide and recombinant CSP protein

Mice were bled two weeks after the last immunization. Antibody titers to air-dried sporozoites were determined by the indirect fluorescent antibody test (IFAT) against air-dried *P. yoelii* sporozoites as described previously (Wang, R. *et al., J. Immunol* **154**:2784-93 (1995); Charoenvit, Y. *et al., J. Immunol.* **146**:1020-5 (1991)). Results were reported in units, defined as reciprocals of the serum dilution at which the OD reading (wavelength 410 nm) is 1.0.

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2.7. Challenge with sporozoites

P. yoelii 17XNL (nonlethal strain), clone 1.1 sporozoites, which were hand dissected from the salivary glands of *Anopheles stephensi*, and suspended in Medium 199 containing 5% fetal bovine serum were used for the challenge. Mice were challenged by intravenous injection of 50 sporozoites in a volume of 0.2 ml. Mice were monitored for blood stage infection from day 5 to day 14 after injection of sporozoites by examining Giemsa-stained thick blood smears. In our experience, mice that are negative on day 14 never become positive, so mice negative on day 14 were considered protected.

3. Results and discussion

3.1. Inhibition of sporozoite invasion of hepatocytes

The antibody levels after administration to C57BL/6 ($H-2^b$) mice of 2 doses of PADRE-PfB and PADRE-PyB peptides in CFA/IFA have now been described (see, e.g., Del Guercio, M.F. *et al., Vaccine* 15:441-8 (1997)). As a foundation for moving to *in vivo* challenge studies we determined whether the antibodies induced by this immunization regimen inhibited sporozoite invasion of hepatocytes *in vitro*. We first assessed the effects of immune sera on invasion and development of *P. falciparum* sporozoites in human hepatocytes. Sera from mice immunized with PyB or PADRE-PyB did not inhibit sporozoite invasion and development (Table 6). The greatest inhibition was by sera from mice immunized with PADRE-PfB (97%). Sera from mice immunized with PfB also gave significant *in vitro* inhibition (80%). This was probably due to the fact that PfB also contains a helper T-cell epitope for $H-2^b$ mice (Good, M.F. *et al., J Exp Med* 164:655-60 (1986); Hoffman, S.L. *et al., Exp Parasitol* 64:64-70 (1987)) and did not require PADRE for induction of antibodies, although the PADRE-PfB-immunized mice did produce higher levels of antibodies, as previously shown (Del Guercio, M.F. *et al., Vaccine* 15:441-8 (1997)).

Table 6

In vitro inhibition of liver stage development in human hepatocytes infected with *P. falciparum* sporozoites.

Sera[a]	Mean schizonts/well + S.D.	% Inhibition
CFA	77 + 4	n.c.[b]
PyB	70 + 14	9.1
PADRE-PyB	84 + 8.5	-9.1
PfB	15.6 + 8.7	79.7
PADRE-PfB	2 + 0.8	97.4

[a]Sera were assessed at a final dilution of 1:100.

[b]n.c. designates the negative control.

5

In the case of *P. yoelii* sporozoites, sera from mice immunized with PfB or PADRE-PfB gave minimal, nonsignificant inhibition (8.5-13.4% at a serum dilution of 1:100) of invasion of mouse liver cells (Table 7). Sera from mice immunized with PyB gave 50% inhibition at this dilution, while mice immunized with PADRE PyB gave significantly greater inhibition (91 and 88% at 1:100 and 1:200 dilutions) (Table 7).

10

In conclusion, the data in Table 6 and Table 7 clearly demonstrate that sera from mice immunized with PADRE-PfB inhibited *P. falciparum* sporozoite invasion of human hepatocytes by 98% and had no significant effect on *P. yoelii* invasion of murine hepatocytes. In contrast, sera from mice immunized with PADRE-PyB inhibited *P. yoelii* sporozoite invasion and development in murine hepatocytes by approximately 90%, but had no significant effect on *P. falciparum* invasion and development in human hepatocytes.

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Table 7

In vitro inhibition of liver stage development in mouse hepatocytes infected
with P. yoelii sporozoite

Sera[a]	Mean schizonts/well + S.D.	% Inhibition
CFA		
1:100	82 + 3.3	[a] n.c.
1:200	81 + 10	n.c.
PyB		
1:100	41 + 3.8	50.0
1:200	46 + 4	43.2
PADRE-PyB		
1:100	7.6 + 1.2	90.7
1:200	96 + 2	88.1
PfB		
1:100	71 + 7	13.4
1:200	76 + 7	6.2
PADRE-PfB		
1:100	75 + 5	8.5
1:200	79 + 6	2.5

[a] n.c. designates the negative control.

5

3.2. PADRE-PyB constructs protect against sporozoite challenge

Encouraged by the data from the experiments shown above, we determined
next if immunization with the PADRE-PyB peptide would protect mice against sporozoite
challenge. In order to select a control immunogen we relied on the following information.
10   We have previously reported that immunization with a multiple antigen peptide branched
chain polymer including the 35 amino acid P2P30 universal T-cell epitope sequences from
tetanus toxin, and four copies of the six amino acid tandem repeat (QGPGAP) from the P.
yoelii circumsporozoite protein (PyCSP) in multiple adjuvants induces high levels of
antibodies that inhibit sporozoite invasion of hepatocytes in vitro and protect against
15   sporozoite challenge in vivo (Wang, R. et al., J. Immunol 154:2784-93 (1995)). We have

also determined that doses of 25 µg of this PyCSP MAP induce higher levels of protection than do higher doses. Accordingly, this immunogen was used as a positive control in our experiments.

The data shown in Table 8 demonstrate that immunization with 100 µg of the linear PADRE-PyB peptide induced levels of antibodies against sporozoites and the PyCSP at least as high as those induced in mice that received 25 µg of the PyCSP-MAP. Immunization with PyB alone was poorly immunogenic. Most importantly, immunization with the simple linear PADRE-PyB peptide in Titermax™ protected 75% of mice against developing blood stage parasitemia after sporozoite challenge. This level of protection was comparable to that induced by immunization with the more complex, and difficult to synthesize and characterize, PyCSP-MAP administered in Titermax™.

Herein, we have demonstrated in the murine model of malaria, represented by infection with *P. yoelii* sporozoites, and the efficacy of simple linear constructs containing dominant B-cell epitopes and PADRE peptides. This type of construct has previously been shown to be highly immunogenic in mice, utilizing various different B-cell epitopes (Del Guercio, M.F. *et al., Vaccine* 15:441-8 (1997)). The antibody response elicited was indistinguishable in magnitude, isotype composition and duration from responses obtained utilizing the same B-cell epitopes in the context of much more complex immunogens such as KLH conjugates, or MAP constructs. Alum or ISA51 adjuvants were effective in delivering these simple PADRE constructs, underlining the potential for further preclinical and clinical development. The potential for clinical development is further underlined by the fact that more complex immunogens, although highly immunogenic and efficacious in model systems, appear to face significant challenges to further development, due to difficulties in analytical and manufacturing processes.

Of note, a *P. falciparum* CSP-MAP including *P. falciparum* B- and T-cell epitopes has been utilized in clinical trials (de Oliveira, G.A. *et al., Vaccine* 12:1012-7 (1994); Moreno, A. *et al., J Immunol* 151:489-99 (1993); Calvo Calle, J.M. *et al., J Immunol* 150:1403-12 (1993)). Recently a number of methods have been proposed for simplifying the synthesis and characterization of MAPs (Rose, K. *et al., Mol Immunol* 32:1031-7 (1995); Zeng, W. *et al., J Pept Sci* 2:66-72 (1996)). However, the data reported herein and our preliminary data in monkeys, indicate that MAPs and linear synthetic peptides with *P.*

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falciparum B-epitopes and PADRE or P2P30 as T-helper epitopes induce nearly comparable antibody levels.

Table 8

Antibodies and protective immunity after immunization
of mice with PyCSP synthetic peptide vaccines

Immunogen/ Adjuvant	Infected/ challenged	% Protected	IFAT Sporozoites (titer[a] x 10-3)	ELISA (QGPGAP)$_2$ PyCS.1 (OD Units x 10-3)[b]	
PyB/Titermax™	7/8	12.5	-	-	-
PADRE-PyB/ Titermax™	2/8	75.0	3.2	25.6	25.6
PyCSP-MAP/ Titermax™	2/7	71.4	3.2	12.8	12.8
-/ Titermax™	7/8	12.5	-	-	-
Infectivity control	8/8	0	ND	ND	ND

[a]Titer is defined as the reciprocal of the last serum dilution yielding positive reactivity as detected by fluorescence microscopy. [b]The reciprocal of the serum dilution at which the optical density (410 nm) was 1.0.

Example 2

***In Vitro* Immunogenicity of Pan DR-Binding Peptides from Table 9**

Introduction

To evaluate the potential of PADRE molecules to provide T cell help, some of the preferred peptides set out in Table 9 were evaluated for their capacity to stimulate *in vitro* T cell responses in PBMC from 5 normal individuals.

Method

Generation of Antigen-Specific T cell Responses from Human PBMCs

PBMC from health donors were stimulated *in vitro* using a protocol adapted from Manca, F., Habeshaw, J. and Dalgleish, A., *J. Immunol.* 146, 1964-1971) (1991). PBMC were purified over Ficoll-Paque (Pharmacia LKB, Uppsala, Sweden) and plated in a 24-well tissue culture plate (Costar, Cambridge, Massachusetts) at 4 x 10^6 PBMC/well. The peptides were added at a final concentration of 10 μg/ml. Cultures were then incubated at 37°C, 5% CO$_2$. On day 4, recombinant interleukin-2 (IL-2) was added at a final concentration of 10 ng/ml. Cultures were fed every 3 days thereafter by aspirating off 1 ml of media and replacing it with fresh medium containing IL-2. Two additional stimulations

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of the T cells with antigen were performed on approximately days 14 and 28. The T cells (3 x 10^5/well) were stimulated with peptide (10 μg/ml) using autologous PBMC cells [2 x 10^6 irradiated [7500 rads]/well] as antigen-presenting cells in a total of three wells of a 24-well tissue culture plate. In addition, on days 14 and 28, T cell proliferative responses

5   were determined as follows: 2 x 10^4 T cells/well; 1 x 10^5 irradiated PBMC/well as antigen-presenting cells; peptide concentration varying between 0.01-10 μg/ml final concentration. The proliferation of the T cells was measured 3 days later by the addition of [^{3}H]thymidine (1 μCi/well)(ICN, Irvine, California) 18 hr prior to harvesting the cells. Cells were harvested onto glass filters (LKB Walla cell harvester 1295-001, Gaithersburg,

10   Maryland), and thymidine incorporation (LKB β plate counter 1205) was measured.

Results and Discussion

        The peptides 965.08, 965.09, 965.10, 965.14, 965.15, 965.16, and 965.17 were evaluated for their capacity to stimulate *in vitro* T cell responses in PBMCs from 5

15   normal individuals (X144, X162, X349, X372, and X460). See Table 10. After two *in vitro* stimulations only peptides 965.10 and 965.17 were able to stimulate T cells significantly, 3.9 and 2.4 times above background. However, by the third stimulation, all 7 peptides stimulated T cells at least 2 times above background. These results indicate that these pan DR epitopes provide T cell help for both humoral and cell-mediated responses.

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Table 9

Binding Activity of PADRE Analogs

PEPTIDE	SEQ ID NO.	SEQUENCE	DR1	DR2wB2	DR3	DR4w4	DR4w14	DR5	DR7	DRw53	DQ3.1
965.08	29	aK(X)VAANTLKAAa-NH$_2$	1.2 (1)	3.8	250	3	13.8	8	192.3	163.8	--
965.09	30	aK(X)VAAYTLKAAa-NH$_2$	0.8	7.4	250	1	7	5.4	192.3	86.4	--
965.10	31	aK(X)VAAWTLKAAa-NH$_2$	1.2	5.6	119	2.8	9.8	11.1	147.1	141.8	25
965.14	32	aK(X)VAAKTLKAAa-NH$_2$	3.6	8	781	7.4	62.5	3.4	227	52.8	--
965.15	33	aK(X)VAAHTLKAAa-NH$_2$	1.9	5.4	1389	3.2	13.8	29.9	156.3	79.2	--
965.16	34	aK(X)VAAATLKAAa-NH$_2$	4.2	6.1	1471	6.2	55.6	16.7	227	131.9	--
965.17	35	AK(X)VAAWTLKAAA-NH$_2$	2	5.9	1786	3.8	26.7	9.1	147.1	169.6	--
553.01	36	QYIKANSKFIGITE	51.5	20	2717	8036	10000	20	25	--	--
553.02	37	qYIKANSKFIGITEa	238	25.3	-- (2)	--	--	83.3	49	--	--

(1) = nM IC$_{50}$ values

(2) dashes indicate >10,000 nM

(X) = cyclohexylalanine

"-NH$_2$" indicates amidation at the carboxyl terminus of the peptides.

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TABLE 10
MAX CPM and STIMULATION INDEX

	X144 (Δmax cpm)	S.I.	X162 (Δmax cpm)	S.I.	X349 (Δmax cpm)	S.I.	X372 (Δmax cpm)	S.I.	X460 (Δmax cpm)	S.I.	AVER- AGE ΔMAX CPM	AVER- AGE S.I.
2nd Stimulation												
965.08	8440	1.3	9234	2.6	9065	1.8	23017	1.6	6286	1.4	11208	1.7
965.09	13693	1.8	10710	1.1	18079	3.8	17813	1.6	0	1	12059	1.9
965.10	11663	1.8	20693	7.9	22133	3.5	44414	3.2	29933	3.3	25767	3.9
965.14	3570	1.1	4655	2.1	4421	1.3	10608	1.4	4365	1.4	5523	1.5
965.15	7179	1.4	8461	3.4	10848	1.8	10011	1.3	3100	1.2	7920	1.8
965.16	4388	1.2	4090	2.1	0	1	736	1	0	1	1843	1.3
965.17	17391	1.7	6323	2	26512	5.6	8471	1.3	4994	1.3	12738	2.4
3rd Stimulation												
965.08	3559	1.4	14622	2.3	31639	2.8	8710	1.9	1820	1.6	12070	2
965.09	14087	2.6	35635	5.2	31811	5.5	29159	4.4	2301	2.9	22599	4.1
965.10	33159	14	34548	11	38931	3.5	36995	15	11376	15	31002	11.5
965.14	4319	2.3	10734	1.7	17849	2	19140	2.1	874	2	10583	2
965.15	13055	2.4	12309	1.6	20653	2.4	30810	4.7	486	1.2	15462	2.5
965.16	5220	1.5	4762	1.9	9316	1.6	10439	1.7	37	1	5955	1.5
965.17	32665	4.1	28065	4.1	31983	4	30661	3.2	3619	2.2	25399	3.5

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It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of

5   this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference for all purposes.

WHAT IS CLAIMED IS:

1.   A composition comprising a peptide that binds to six or more different members of a panel of HLA-DR molecules, wherein the peptide comprises at least about nine amino acids, said peptide having the formula Z_n-$X_1X_2X_3X_4X_5X_6X_7X_8X_9$-$Z_c$, wherein:

        X_1 is an amino acid selected from the group consisting of: (X), Y, F, M, L, I, V, and W, wherein (X) is cyclohexylalanine;

        X_2 is an amino acid selected from the group consisting of: I and V;

        X_3, X_4, X_5, X_7, X_8, and X_9 are each an amino acid;

        X_6 is an amino acid selected from the group consisting of: T, V, M, S, A, C, P, L, and I;

        Z_n and Z_c each comprise 1 to about 15 amino acids.

2.   The composition of claim **1**, wherein either or both of Z_n and Z_c comprise at least one D-amino acid.

3.   The composition of claim **1**, wherein Z_n comprises K or A adjacent to X_1; X_1 is (X), Y or F; and X_2 is I or V.

4.   The composition of claim **1**, wherein the peptide inhibits activation of a T cell which displays a receptor which is specific for a DR molecule and X_5 is a non-polar, non-charged, non-bulky, non-aromatic amino acid.

5.   The composition of claim **4**, wherein X_5 is A.

6.   The composition of claim **4**, wherein one or more of X_3, X_4, X_7, X_8, and X_9 is a non-polar, non-charged, non-bulky, non-aromatic amino acid.

7.   The composition of claim **6**, wherein Z_n comprises at least one non-polar, non-charged, non-bulky, non-aromatic amino acid.

1   **8.**   The composition of claim **6**, wherein one or more of X_3, X_4, X_7, X_8, and

2   X_9 is A.

1   **9.**   The composition of claim **1**, wherein the peptide stimulates activation of

2   a T cell which displays a receptor specific for a DR molecule and wherein X_5 is a polar,

3   charged, bulky, or aromatic amino acid.

1   **10.**   The composition of claim **9**, wherein X_5 is W.

1   **11.**   The composition of claim **9**, wherein one or more of X_3, X_4, X_7, X_8, and

2   X_9 is a polar, charged, bulky, or aromatic amino acid.

1   **12.**   The composition of claim **11**, wherein one or more of X_3, X_4, X_7, X_8,

2   and X_9 is W.

1   **13.**   A composition comprising a peptide that binds to six or more different

2   members of a panel of HLA-DR molecules, wherein the peptide comprises at least about

3   nine amino acids, said peptide having the formula Z_n- $X_0X_1X_2X_3X_4X_5X_6X_7X_8X_9$-$Z_c$,

4   wherein:

5   X_0 is an amino acid selected from the group consisting of: K and A;

6   X_1 is an amino acid selected from the group consisting of: (X), Y and F;

7   X_2 is an amino acid selected from the group consisting of: I and V;

8   X_3, X_4, X_5, X_7, X_8, and X_9 are each an amino acid;

9   X_6 is T;

10   Z_n and Z_c each comprise 1 to about 15 amino acids.

1   **14.**   The composition of claim **13**, wherein either or both of Z_n and Z_c

2   comprise at least one D-amino acid.

1   **15.**   The composition of claim **13**, wherein the peptide inhibits activation of

2   a T cell which displays a receptor specific for a DR molecule and wherein X_5 is a non-polar,

3   non-charged, non-bulky, non-aromatic amino acid.

1   **16.**   The composition of claim **15**, wherein X_5 is A.

1   **17.**   The composition of claim **15**, wherein one or more of X_3, X_4, X_7, X_8

2   and X_9 is a non-polar, non-charged, non-bulky, non-aromatic amino acid.

1   **18.**   The composition of claim **15**, wherein either or both of Z_n and Z_c

2   comprises at least one non-polar, non-charged, non-bulky, non-aromatic amino acid.

1   **19.**   The composition of claim **13**, wherein the peptide stimulates activation

2   of a T cell which displays a receptor specific for a DR molecule and wherein X_5 is a polar,

3   charged, bulky, or aromatic amino acid.

1   **20.**   The composition of claim **19**, wherein X_5 is W.

1   **21.**   The composition of claim **19**, wherein one or more of X_3, X_4, X_7, X_8,

2   and X_9 is a polar, charged, bulky, or aromatic amino acid.

1   **22.**   The composition of claim **21**, wherein one or more of X_3, X_4, X_7, X_8,

2   and X_9 is W or K.

1   **23.**   The composition of claim **22**, wherein one or more of X_7 and X_8 is K.

1   **24.**   The composition of claim **1**, wherein the panel of HLA-DR molecules

2   comprises at least six members selected from the group consisting of DR1, DR2w2b,

3   DR2w2a, DR3, DR4w4, DR4w14, DR5, DR6, DR7, DR8, DR9, DR52a, DR52b, DR52c

4   and DR53.

1   **25.**   The composition of claim **1**, wherein the peptide is a pan-DR peptide
2   that binds to at least about seven members of the panel with high affinity.

1   **26.**   The composition of claim **1**, wherein the composition comprises a
2   pharmaceutically acceptable carrier.

1   **27.**   The composition of claim **1**, which further comprises a CTL inducing
2   peptide.

1   **28.**   The composition of claim **27**, wherein the CTL inducing peptide is
2   acetylated, palmitylated, or acylated with a fatty acid.

1   **29.**   The composition of claim **27**, wherein the CTL inducing peptide is
2   linked to the peptide to form a CTL/T helper peptide conjugate.

1   **30.**   The composition of claim **29**, wherein the CTL/T helper peptide
2   conjugate is linked to a carrier.

1   **31.**   The composition of claim **29**, wherein the CTL inducing peptide is
2   linked to the peptide by a spacer molecule.

1   **32.**   The composition of claim **1**, wherein the peptide is a naturally occurring
2   peptide that arises upon processing of a protein by an antigen-presenting cell.

1   **33.**   The composition of claim **32**, wherein the peptide is a human peptide.

1   **34.**   The composition of claim **1**, wherein the peptide is linked to one or
2   more carbohydrate epitopes and the composition can induce an immune response to an
3   antigenic carbohydrate.

1   **35.**   The composition of claim **34**, wherein the carbohydrate epitope

2   comprises an antigenic determinant from a bacterium, a virus, a cancer cell, a fungus, or a

3   parasite.

1   **36.**   The composition of claim **34**, wherein the carbohydrate epitope is

2   linked to the carboxy-terminus of the peptide.

1   **37.**   The composition of claim **34**, wherein the carbohydrate epitope is

2   covalently linked to the PADRE peptide through a linker.

1   **38.**   The composition of claim **37**, wherein the linker comprises a cysteine

2   residue.

1   **39.**   The composition of claim **37**, wherein the linker consists of an

2   aminocaproic acid residue and a cysteine residue.

1   **40.**   The composition of claim **34**, wherein the peptide is further linked to a

2   surface-active material.

1   **41.**   The composition of claim **40**, wherein the surface-active material is a

2   lipid moiety, a polymer, polyalkylene glycol, or a surfactant.

1   **42.**   The composition of claim **40**, wherein the surface-active material is

2   linked to the N-terminus of the PADRE peptide.

1   **43.**   The composition of claim **40**, wherein the surface-active material

2   comprises palmitic acid.

1   **44.**   The composition of claim **43**, wherein the surface-active material is a

2   lipid moiety which is PAM_2K, wherein K is a lysine residue and PAM is a palmitic acid

3   residue.

1   **45.**   A peptide that binds to seven or more members of a panel of HLA-DR

2   molecules, the peptide comprising a neutral amino acid at each position that is not a critical

3   contact site, which critical contact sites are necessary for binding of the antigen to a selected

4   HLA-DR molecule.

1   **46.**   A method for identifying a pan-DR peptide, the method comprising

2   analyzing one or more peptide sequences for a peptide that has the formula Z_n-

3    $X_1X_2X_3X_4X_5X_6X_7X_8X_9$-$Z_c$, wherein:

4   X_1 is an amino acid selected from the group consisting of: (X), Y, F, M,

5   L, I, V, and W, wherein (X) is cyclohexylalanine;

6   X_2 is an amino acid selected from the group consisting of: I and V;

7   X_3, X_4, X_5, X_7, X_8, and X_9 are each an amino acid;

8   X_6 is an amino acid selected from the group consisting of: T, V, M, S,

9   A, C, P, L, and I;

10   Z_n and Z_c each comprise 1 to about 15 amino acids; and

11   selecting a peptide or peptides that have such formula.

12   .

1   **47.**   The method of claim **46**, further comprising the step of testing the

2   selected peptide or peptides for binding to three different members of a panel of HLA-DR

3   molecules that comprises at least three members selected from the group consisting of DR1,

4   DR2w2b, DR2w2a, DR3, DR4w4, DR4w14, DR5, DR6, DR7, DR8, DR9, DR52a, DR52b,

5   DR52c, and DR53.

1   **48.**   The method of claim **46**, wherein the selected peptide is a naturally

2   occurring peptide.

1   **49.**   The method of claim **46**, wherein the peptides are tested for binding to

2   sequential panels of HLA-DR molecules.

1   **50.**   The method of claim **49**, wherein the sequential panel comprises a

2   primary panel which comprises DR1, DR4w4 and DR7 HLA molecules.

1   **51.**   The method of claim **50**, wherein peptides that bind to at least two

2   members of the primary panel are tested for binding to members of a secondary panel which

3   comprises DR2w2β1, DR2w2β2, DR6w19 and DR9 HLA molecules.

1   **52.**   The method of claim **51**, wherein peptides that bind to at least three

2   members of the secondary panel are tested for binding to members of a tertiary panel which

3   comprises DR4w15, DR5w11 and DR8w2 HLA molecules.

1   **53.**   A method for rational design of a peptide that binds to three or more

2   different members of a panel of HLA-DR molecules, which peptide has the formula Z_n-

3    $X_1X_2X_3X_4X_5X_6X_7X_8X_9$-$Z_c$, the method comprising:

4   introducing at position X_1 an amino acid selected from the group

5   consisting of (X), Y, F, M, L, I, V, and W, wherein (X) is cyclohexylalanine;

6   introducing at position X_2 an amino acid selected from the group

7   consisting of I and V; and

8   introducing at position X_6 an amino acid selected from the group

9   consisting of T, V, M, S, A, C, P, L, and I;

10   wherein X_3, X_4, X_5, X_7, X_8, and X_9 are each an amino acid; and

11   Z_n and Z_c each comprise 1 to about 15 amino acids.

1   **54.**   The method of claim **53**, wherein the method further comprises testing

2   the peptide to identify those that bind with high to intermediate affinity to three or more

3   different members of a panel of HLA-DR molecules.

1   **55.**   The method of claim **53**, wherein the panel of HLA-DR molecules

2   comprises at least three members selected from the group consisting of DR1, DR2w2b,

3   DR2w2a, DR3, DR4w4, DR4w14, DR5, DR6, DR7, DR8, DR9, DR52a, DR52b, DR52c,

4   and DR53.

1   **56.** The method of claim **53**, wherein the peptide is a pan-DR peptide that
2   binds to at least about three members of the panel with high affinity.

1   **57.** The method of claim **56**, wherein the pan-DR peptide binds to at least
2   about three members of the panel with an IC_{50} of about 500 nM or less relative to the IC_{50} of
3   a reference peptide.

1   **58.** The method of claim **53**, wherein the method further comprises
2   rationally designing the peptide to inhibit DR-restricted T cell proliferation by replacing one
3   or more amino acids at positions X_3, X_4, X_5, X_7, X_8, and X_9 with a non-polar, non-charged,
4   non-aromatic, non-bulky amino acid.

1   **59.** The method of claim **58**, wherein the non-polar, non-charged, non-
2   aromatic, non-bulky amino acid is selected from the group consisting of Ala, Gly, and Pro.

1   **60.** The method of claim **59**, wherein the non-polar, non-charged, non-
2   aromatic, non-bulky amino acid is Ala.

1   **61.** The method of claim **58**, wherein each amino acid at positions X_3, X_4,
2   X_5, X_7, X_8, and X_9 is replaced with a non-polar, non-charged, non-aromatic, non-bulky
3   amino acid.

1   **62.** The method of claim **58**, wherein the method further comprises:
2   identifying those test peptides that retain their ability to bind with high
3   to intermediate affinity to greater than 50% of members of a panel of HLA-DR molecules;
4   and
5   testing the peptides that retain HLA-DR binding ability to identify those
6   that can inhibit DR-restricted T cell proliferation.

1   63.   The method of claim **62**, wherein the testing of the peptides to identify
2   those that can inhibit DR-restricted T cell proliferation is conducted in an *in vitro* assay
3   system or in an *in vivo* system.

1   64.   A peptide that binds to greater than 50% of members of a panel of
2   HLA-DR molecules, wherein the peptide is identified using the method of claim **53**.

1   65.   The method of claim **53**, wherein the method further comprises
2   rationally designing the peptide to induce an immune response by including at one or more
3   of positions X_3, X_4, X_5, X_7, X_8, and X_9 a polar, charged, aromatic, or bulky amino acid.

1   66.   The method of claim **65**, wherein the polar, charged, aromatic or bulky
2   amino acid is selected from the group consisting of W, K, and (X).

1   67.   The method of claim **65**, wherein positions X_7 and X_8 are each
2   independently selected from the group consisting of W and K.

1   68.   The method of claim **65**, wherein the method further comprises:
2   identifying those test peptides that retain their ability to bind with high
3   to intermediate affinity to greater than 50% of members of a panel of HLA-DR molecules;
4   and
5   testing the pan-DR peptides that retain HLA-DR binding ability to
6   identify those that can induce an immune response.

1   69.   The method of claim **68**, wherein the amino acid is selected from the
2   group consisting of cyclohexylalanine, tryptophan, and lysine.

1   70.   The method of claim **68**, wherein the immune response is a humoral
2   response or a cytotoxic response.

1   71.   The method of claim **68**, wherein the peptides are tested *in vivo*.

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1   **72.**   A peptide that comprises a pan-DR T helper epitope that binds to three

2 or more different members of a panel of HLA-DR molecules, wherein the peptide is

3 obtained using the method of claim **68**.

1   **73.**   A method of inhibiting antigen-specific activation of T cells, the method

2 comprising contacting a composition that comprises antigen presenting cells and the T cells

3 with a peptide that binds to three or more different members of a panel of HLA-DR

4 molecules, wherein:

5   the antigen presenting cells display a member of the panel of HLA-DR

6 molecules and the T cells are of the DR MHC restriction; and

7   at least one amino acid residue in the peptide which is not a critical

8 contact site for binding to the HLA-DR molecule but is involved in binding to a T cell

9 receptor is substituted with a neutral amino acid.

1   **74.**   The method of claim **73**, wherein the method comprises administering

2 to a patient a therapeutically effective dose of a pharmaceutical composition that comprises

3 the peptide and a pharmaceutically acceptable carrier.

1   **75.**   A method of inducing antigen-specific activation of T cells, the method

2 comprising contacting a composition that comprises antigen presenting cells and the T cells

3 with a peptide that binds to three or more members of a panel of HLA-DR molecules,

4 wherein the antigen presenting cells display a member of the panel of HLA-DR molecules

5 and the T cells are of the DR MHC restriction, and further wherein the peptide has at least

6 one characteristic selected from the group consisting of:

7   at least one amino acid which does not constitute a critical contact site

8 in a pan-DR peptide from which the peptide is derived is substituted with an amino acid

9 having a side chain that has one or more properties selected from the group consisting of

10 increased bulk, hydrophobicity, aromaticity and charge compared to the substituted amino

11 acid;

12   the peptide is conjugated to a CTL-inducing antigenic determinant; and

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13   the peptide is conjugated to a humoral response-inducing antigenic

14   determinant.

1   **76.**   The method of claim **75**, wherein the CTL-inducing antigenic

2   determinant or the humoral response-inducing antigenic determinant comprises a

3   carbohydrate epitope.

1   **77.**   An isolated nucleic acid molecule that encodes a peptide of claim **1**.

INDUCTION OF IMMUNE RESPONSE AGAINST DESIRED DETERMINANTS

ABSTRACT OF THE DISCLOSURE

This invention provides HLA-DR binding peptides, called "pan DR binding peptides" that are recognized by a broad spectrum of DR molecules. These pan DR binding peptides can be used as potent inhibitors or immunogens for class II restricted T cells, as useful human peptide-based immunosuppressants and to provide T cell help for vaccines or other therapeutic agents.

SF 1154722 v1

DECLARATION

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **INDUCTION OF IMMUNE RESPONSE AGAINST DESIRED DETERMINANTS** the specification of which __X__ is attached hereto or _____ was filed on _____ as Application No. _____ and was amended on _____ (if applicable).

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56. I claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Country	Application No.	Date of Filing	Priority Claimed Under 35 USC 119

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date
60/010,510	1/24/96

I claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Date of Filing	Status
08/788,822	1/23/97	pending
09/310,462	5/12/99	pending
08/485,218	6/7/95	abandoned
08/305,871	9/14/94	U.S. Patent No. 5,736,142
08/121,101	9/14/93	abandoned

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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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